Acta

OTO-LARYNGOLOGICA

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EDITOR: C-A. HAMBERGER · STOCKHOLM

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STOCKHOLM, SWEDEN

Conferences and Meetings

1978, Sept 21–24 First International Course on Multichannel Cochlear Implant to be held in Paris Addr Pr C.-H Chouard, Hôpital Saint Antoine 184, rue de Faubourg Saint Antoine, 75012 Paris

1978, Sept 25–30 The IVth Congress of the European Association for Maxillo-Facial Surgery (E A M F S) will be held in Vicenza, Italy Addr Pavillon de Chirurgie Maxillo-Facial et de Odontostomatologie Hôpital Régional S Bortolo", 36100 Vicenza, Italy

1978, Sept 25–29 The Third International Congress on Noise as a Public Health Problem Biological and Behavioral Effects, is scheduled to be held in Freiburg West Germany Addr Int Commission on Biological Effects on Noise Universitäts klinikum, Johannes Gutenberg Universitat Obere Zahlbacher Strasse 67, D-6500 Mainz, BRD

1978, Oct 2–7 Cours Pratique de Septo-Rhinoplastie Fonctionnelle et de Rhino-Sinuscopie donné à l Université Libre de Bruxelles Addr Docteur Jacques Willemot, Luitenant Willemotlaan 90, B-9910 Mariakerke Gent, Belgique.

1978, Oct 5–7 1st European Course in Pediatric Otolaryngology will be held in Trieste, Italy Addr Prof R Fior, Istituto per l Infanzia, Via dell Istria 65, Trieste, Italy

1978, Oct 16–19 Cours Vestibulométrie Clinique donné en Strasbourg, France Addr Clinique O R L., Hospices Civils de Strasbourg, 1 Place de l Hôpital, 67005 Strasbourg-Cedex

1978, Oct 30–Nov 1 Society for Neuroscience Eighth Annual Meeting will be held in Pittsburgh, PA Addr Dr T Gualtierotti, University of Pittsburgh, Vestibular Research Laboratory, 207 Hill Building, 3434 Fifth Avenue Pittsburgh, PA 15260

1978, Oct 29 – Nov 3 The Vth International Conference on Pneumoconioses will be held in Caracas, Venezuela Addr International Labour Office, Occupational Safety and Health Branch CH 1211 Geneva 22, Switzerland

1978 Oct 29–31 Semi Annual VII Nerve Surgical Dissection Course of the New York Medical College will be held at Westchester County Medical Center Addr Mrs P Tamkin, c/o Dr P Guibor, 630 Park Avenue New York, New York 10021

1978, Oct 30–Nov 10 A Two-Week Temporal Bone Surgical Dissection Course will be held at the Ear Research Institute in Los Angeles Addr Antonio de la Cruz, 256 South Lake Street, Los Angeles, CA 90057

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Brown, A. 1953 Obliterative frontal sinusitis. *Ann Otol 62*, 377

— 1954 *Arthritis and Rheumatoid Conditions* John Wiley, New York

Brown, A & Smith, B 1956 On the chemistry of the endolymph. *Acta Otolaryngol* (Stockh) *46*, 408

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Acta Otolaryngol 86 1–8, 1978

RESPONSES OF AUDITORY NERVE FIBRES TO NOISE STIMULI SHOW COCHLEAR NONLINEARITIES

Aage R Møller

From the Division of Physiological Acoustics Department of Environmental Medicine Karolinska Institutet Stockholm Sweden

(Received October 6 1977)

Abstract The frequency selectivity of the peripheral auditory frequency analyser was determined on the basis of recordings from single auditory nerve fibres in the rat using pseudorandom noise as stimulus The impulse response function was arrived at by cross-correlating period histograms of the recorded activity with the noise Frequency transfer functions were computed by Fourier transformation of the derived impulse response function The impulse response function was observed to become shorter with increasing stimulus intensity A concomitant widening of the frequency transfer function and a downward shift in the centre frequency were seen Since the method used is insensitive to nonlinearities the derived impulse and frequency response functions are assumed to be valid approximations of the basilar membrane properties The results then support the hypothesis that the basilar membrane is a nonlinear frequency analyser whose selectivity decreases as sound intensity increases

The model generally accepted for the peripheral auditory analyser is a band pass filter complemented by a rectifier a lowpass filter and a triggering device The band pass filter consists of the frequency selectivity of the basilar membrane the rectifier is the unidirectionality of the sensory hair cells and the low pass filter is the smoothing action of the neural excitatory process that transduces the vibration of the hair cells into nerve impulses in the axons of the auditory nerve (Weiss 1966)

A fundamental question is whether the frequency selectivity of the auditory periphery is solely a result of mechanical tuning of the basilar membrane The first quantitative data on basilar membrane tuning originate from v Bekesy who measured the displacement of the basilar membrane of human cadaver ears in the low frequency region of the basilar membrane These results represented a great step forward in auditory physiology but at the same time they emphasized the need of an additional mechanism in order to explain the great frequency selectivity of the ear When it became possible to measure basilar membrane vibration at lower sound intensities and in anesthetized animals, as has been done by Rhode (1971), Johnstone et al (1970) and Wilson & Johnstone (1972), greater frequency selectivity values (narrower tuning curves) were obtained than those presented by v Bekesy The obtained mechanical tuning curves of the basilar membrane were, however, wider than frequency threshold curves of single auditory fibres Such 'frequency tuning curves (FTC), show the threshold of single nerve fibres in response to pure tones as a function of frequency while the mechanical tuning curves show the vibration amplitude at or above 70 dB SPL (Rhode, 1971) A fundamental question is whether this discrepancy in frequency selectivity is due to the fact that the vibration of the basilar membrane is nonlinear in such a way that the selectivity increases as sound intensity is decreased

The observation by Rhode (1971, 1973) shows that the frequency selectivity of the basilar membrane in the squirrel monkey decreases with sound intensity between 70 and 90 dB SPL That could account for the dis

Present address Eye & Ear Hospital University of Pittsburgh School of Medicine Pittsburgh Pa 15213 USA

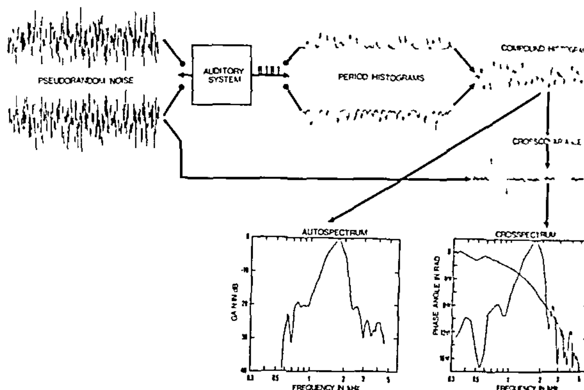


Fig 1 Schematic illustration of the data processing

crepancy between neural and mechanical tuning However, other studies using similar or rent methods (Johnstone et al , 1970, Wil- & Johnstone, 1972) for measuring basilar brane vibration in the guinea pig have ailed to show such nonlinear motion

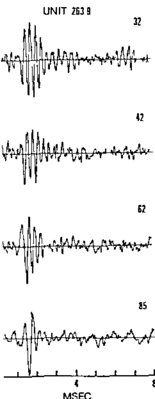
Various types of mechanical transformations of the motion of the basilar membrane are therefore still further explanations which might be proposed for the discrepancy in the sharpness of the frequency tuning observed between the neural tuning curves and the motion of the basilar membrane The basilar membrane motion has always been measured in terms of up and down movement It is not certain that the up and down movement of the basilar membrane is the most efficient stimulus for the hair cells

Evans (1975) assumes another explanation He developed the hypothesis that there is a sharply tuned (presumably neural) band pass filter succeeding the relatively broadly tuned cochlear (mechanical) filter He found that the existence of such a "second filter" was sup

mals with normal cochleas (Kiang et al , I Evans, 1974*a*, *b*) Evans (1975) thereby cluded that the neural threshold tuning cu were sharper and narrower than the mech cal tuning curves of the basilar memb owing to this "second filter"

The technical difficulties inherent in m uring the basilar membrane motion directl physiological sound levels make it appealir use neurophysiological methods to deterr basilar membrane displacement, whereby hair cells serve as "sensors" of the ba membrane vibration

Kiang et al (1965) showed that peristim time histograms of the response of prim fibres with a low characteristic frequency a periodic pattern, assumed to reflect damped oscillation of the basilar membra Unfortunately, such results do not lend th selves to a quantitative analysis, mainly d the influence on the responses of the re



? Typical cross-correlograms representing the im response function of a typical nerve fibre The lus intensities (dB SPL measured in 1/3 octave s) are indicated by caption numbers The threshold of nit was about 15 dB SPL The amplitude of the cor rams is normalized to the same maximal value

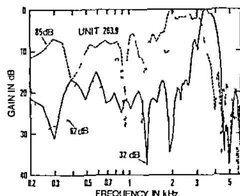


Fig 3 Cross spectra of the responses of the unit depicted in Fig 2 representing the frequency domain transfer functions The cross spectra are normalized to the same maximal value

properties of the neurone involved The linearity resulting from the neural trans tion in the hair cells also influences the ilts

ioblick & Pfeiffer (1969) further developed above mentioned method and introduced a cellation procedure to disclose nonlineari in the inner ear They combined the his rams of the responses to clicks of one po- ty with those of the responses to the clicks the reverse polarity, which yielded a com ind histogram assumed to be an approxima n of the basilar membrane in both direc- ns Although this method provided impor t information about the nonlinearities in the chlea, it did not enable the determination of : impulse response function of the basilar :mbrane Other studies (Møller, 1970) have shown that the frequency selectivity represented by neural tuning curves is available immediately A neural sharpening involving conventional chemical synaptic transductions can therefore be excluded since that would result in delays on the order of at least 0 7 msec

Quantitative information about the spectral acuity of the auditory frequency analyser at different stimulus intensities was hence lacking

The discharge pattern of single auditory fibres in response to noise stimuli can provide quantitative information about the spectral selectivity of the auditory periphery De Boer (1969, 1970), using a reverse correlation technique, found that the frequency selectivity of single primary nerve fibres of the cat in response to band pass filtered noise was similar to the frequency threshold curves (FTC) obtained in response to pure tones

The reverse correlation technique is similar to cross correlating the output of a system with the input When the input is a random noise with a uniform spectrum in the frequency range where the system under test has significant transmission, the cross correlation function is a valid approximation of the system s impulse response The results are essentially independent of nonlinearities (memoryless) that follow the frequency selective filter of the system When this method is

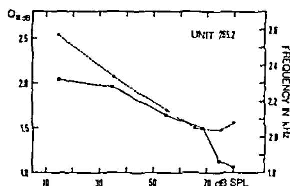


Fig 4 Relative width (Q_{10dB} ●---●) and center frequency (■—■) of the transfer function plotted as a function of the sound level (in dB SPL measured in 1/3 octave bands)

applied to a system in which the input first enters a linear band pass filter and then a non linear device the results largely reflect the linear properties of the system If it can be assumed that the discharge rate of single auditory nerve fibres is a nonlinear transformation of the vibration of the basilar membrane this method would thus yield primarily the linear properties of the membrane including the organ of Corti It is also necessary that the non ... of the neural transduction can be re... to be memoryless Using continuous noise also renders the obtained latency values independent of the stimulus intensity (Møller, 1975) compared with using click stimulation where the latency is a function of the sound level

In the present study the impulse response function of the peripheral auditory analyser was determined on the basis of the responses of single auditory nerve fibres in the anesthetized rat

METHODS

White rats weighing 200–300 g anesthetized with Urethan (1 5 g/kg bw) were used The occipital part of the skull was opened and parts of the cerebellum were removed by suction to render the cochlear nucl...s visible The pinnae were deflected and the ear canal cut after which the head was fi...d in the head holder with hollow earbars F... r details on this procedure were given in previous p... tions (Møller, 1969) Recordings were from single auditory nerve fibres using fine glass pipettes filled with 2 5 mol... sium acetate and with a resistance of ... MΩ The electrodes were placed in c... with the surface of the dorsal cochlear n... aiming at the auditory nerve s entrance... skull cavity (Møller, 1976) Pseudor... noise was used as stimulus It was ger... by a Hewlett Packard noise generator... 3722 A) and applied to a condenser ... phone (Bruel & Kjær type 4131) via c... tional amplifiers and attenuators Th... denser microphone was connected to t... low earbars of the headholder (see ... 1969) The noise generator was set to a... period of 3 33 μs and a sequence le... 4 095 points, i e each noise period had ... tion of 13 64 msec The noise was lo... filtered with an upper cut off frequency... kHz (see Møller, 1974 1976 1977)

Period histograms were produced ... by an Intertechnique DIDAC 800 sy... nized with the periodicity of the noise ... width 20 μs) The polarity of the noi... reversed during the recording every ... and the summation of the discharge... changed to subtraction to produce com...

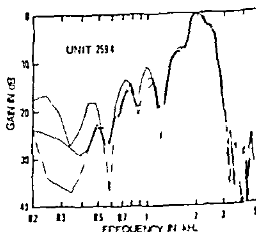
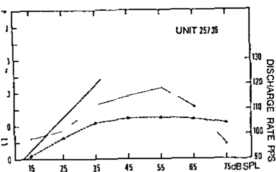
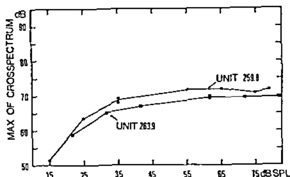


Fig 5 Cross spectra of the responses of the sa... immediately after the unit was encountered a... and 70 minutes later The sound intensity was ... SPL and each curve is based on 2 minutes of da...





6A Average discharge rate (O O) and relative k value of the computed cross spectra (▲---▲) as a ction of stimulus sound level (in dB SPL measured 1/3 octave bands)

Fig 6B Relative peak value of the cross spectra of two other units

tograms (see Fig 1) The histograms were en processed off-line to produce auto-covari

produce auto, cross and coherence tra (see Fig 1 and Møller, 1974, 1976)

RESULTS

results of the present study are based on rdings from 52 fibres Fig 2 shows typical ss correlation functions estimated from dis rges in response to noise with intensities ging from just above threshold (32 dB SPL) 5 dB SPL (measured in a 1/3 octave band) cross correlation functions were normal- d to the same peak value

The duration of these correlation functions een to decrease as the sound intensity in- ases, indicating that the bandwidth of the litory analyser is narrower for low than for h intensity sounds The computed frequen transfer functions (cross spectra) in Fig 3 w even more clearly that the tuning curve vider at high stimulus intensity than at low addition to the widening of the tuning curve, centre frequency is shifted downwards as intensity is increased

The quantitative relationship between the ative width of the peak of the frequency nsfer function and the sound intensity is seen in Fig 4, where the centre frequency is also plotted The relative width is expressed as Q_{10dB}, i e, the ratio between the centre frequency and the bandwidth measured at 10 dB below the peak

The Q-values are observed to decrease monotonically throughout the entire range of intensities studied, i e, from about 10 dB above threshold to more than 80 dB above threshold The centre frequency is also seen to decrease monotonically within about the same range of intensities

The cross spectra (transfer functions) seen in Fig 3 are normalized with regard to the peak values amplitudes Fig 5 shows the peak value of the transfer function as a function of the stimulus intensity The peak value of the transfer function thus determined is a measure of the average degree of phase locking of the discharges to the sound stimulus filtered by the basilar membrane The peak value of the transfer function is noted to reach a plateau at only about 30 dB above the threshold after which it remains virtually constant throughout the intensity range studied Fig 5 also depicts the average discharge rate as a function of the stimulus intensity This curve follows a course that is somewhat different from that of the peak value of the transfer function

These results, typical for units with a centre frequency between 1 and 4 kHz, show that the peripheral auditory analyser functions as a nonlinear spectrum analyser in response

to noise stimuli Since the cross-correlation method used is assumed to be uninfluenced by nonlinearities occurring after the frequency selective elements, the nonlinearity is not likely to originate from the neural transduction process Its source is therefore assumed to be in some activity which would take place prior to the mechanical excitation of the hair cells The nonlinearity found in the present study closely resembles those shown by Rhode in the vibration of the basilar mem brane (Rhode, 1971, 1973) in the intensity range from 70 to 90 dB SPL It may therefore be inferred that the nonlinearities in the pres ent study also originate in the motion of the basilar membrane Moreover, the present results indicate that the nonlinearity extends to lower intensities, thereby bringing about a narrowing of tuning towards lower sound levels Near threshold the frequency transfer functions obtained using noise stimuli are nearly identical to frequency threshold tuning curves obtained using pure tones as stimuli It seems ...u as though the selectivity in single audi... nerve fibres, at least in a first approxima... ...on may be explained on the basis of the vibration of the basilar membrane Results of earlier studies, showing that the full frequency selectivity of auditory neurons is available with no measurable delay (Møller, 1970), are thus supported The sharp tuning seen at low intensities is likely to have little to do with a neural sharpening of a broad mechanical tun ing of the basilar membrane

Finally, it may be inferred from the present results that the waveform of the filtered ver sion of a broadband stimulus is reproduced in the discharge pattern of single auditory nerve fibres within a much larger intensity range (more than 60 dB) than that within which the average discharge rate is a function of the in tensity of a continuous t...n The mean dis charge rate usually reache... ...turation level at only about 20–30 dB a...o... threshold In other studies the discharge ... ...l single audi tory nerve fibres has usual... ...n found to saturate only 20–30 dB above t... threshold These findings, too, are consistent with t... sults of the present study

The dynamic range of the method use... obtaining the cross-spectra is estimated slightly more than 20 dB Thus spectral ... below 20–25 dB are not really significant meaning of the finer details in the com... cross spectra (peaks and valleys) seen ... cularly below the frequency of the maxi... is not clear However, the reproducibil... these cross-spectra is good, as illustrat... Fig 5 where the cross-spectra obtained the same fibre in three successive recor... are shown Even the peaks below the ...quency of the maximum value are reprod... in the spectra computed from all thes... cords

The results graphed in Fig 6 indicate the number of phase locked discharges ... markably constant in the larger part o... dynamic range of the auditory system phase-locked responses of auditory ner... bres are thus to be regarded as an ampli... compressed version, and heavily so, o... motion of the basilar membrane How... there is little information as to the abs... values of the sound intensity in the disch... of primary fibres Nonetheless, such an a... tude-compressed version of the basilar m... brane motion seems extremely well suite... basis for temporal analysis Basilar memb... filtering, in the frequency range where p... locking is prominent, may be assumed to mainly to separate spectral compon... whereupon masking is avoided as a prer... site for a temporal analysis According t... line of reasoning, it seems as though tem... analysis may be more important than spe... as a basis for frequency discrimination i... peripheral auditory system If such is the ... perhaps the cochlea does not take on its as a spectral analyser for frequency disc... nation until we are dealing with frequ... ranges beyond that within which phase l... ing is prominent

Decoding of spatial frequency inform... requires that the nerve fibres be identif...

: higher auditory centre with regard to *average* discharge rate while decoding of oral frequency information calls for the mination, somewhere in the central nerv system, of the modulation of the intervals een individual discharges We do not v how the periodicity information of a pe- c sound is extracted, but neurophysio al evidence indicates that the differences me required for a sound to reach the two can be extracted It is possible that a lar principle is followed in analysis of oaural periodicities whereby the phase-ed responses in individual auditory nerve :s would serve as a basis for this analysis result would then be a decoding into an- r form elsewhere in the auditory nervous em (see Møller, 1978)

the present results, obtained in rats as were, can be regarded as representative the human auditory system it seems as igh the basic principles for frequency anal in the inner ear differ depending upon ther the frequency is above or below 4–5 *In this connection, it is worth noting* the spectral components of human speech the important factor for discrimination be 4 kHz It is likely, then, that both formant quencies and the fundamental frequencies vowels and other voiced sounds are deter ied on the basis of the temporal analysis in inner ear

n patients with hearing loss due to cochlear nage, Pickett & Danaher (1973) have shown it the ability to discriminate small changes the frequency of the second formant (F_2) a two-formant synthetic vowel was im red while the ability to discriminate fre ency changes in a single formant with the ne centre frequency as F_2 was nearly nor il They suggested that this decrease in fre- ency discrimination may be due to an in eased degree of masking from the first for int resulting from the cochlear damage On e basis of the results of the present study, may then be inferred that a broadening in e basilar membrane tuning increases the masking of information, when we are dealing with low frequencies, whereby temporal anal ysis is impaired

Discrimination of sound intensity cannot be explained on the basis of the above described model Since the dynamic range of single nerve fibres with regard to mean discharge rate as well as number of phase locked dis charges is small, only about 20 dB, information on sound intensity throughout the entire audi- tory range cannot be communicated by the discharge rate of individual fibres alone In tensity information needs only a few nerve elements and may be taken care of by e g nerve cells receiving input from a large num ber of auditory nerve fibres and located in higher brain nuclei, such as the cochlear nu cleus Through integration of the output of many primary fibres, information about the absolute sound intensity may be obtained

ACKNOWLEDGEMENT

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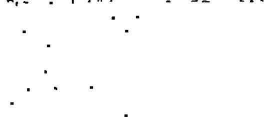
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ZUSAMMENFASSUNG

Die Frequenzselektivität des peripheren Frequenza sators des inneren Ohres wurde an Hand von M elektrodenmessungen von einzelnen Fasern des N a cus der Ratte bestimmt Als Reiz diente pseudo-s stisches Rauschen Die Impulsantwort wurde Kreuzkorrelation der Periodenhistogramme der a zeichneten Aktivität mit dem Rauschen erhalten M von Fourier Transformation der Impulsantwort w sodann die Frequenzubertragungsfunktionen berec



stutzen damit die Hypothese daß die Basilarmen einen nichtlinearen Frequenzanalysator darstellt d Selektivitat mit wachsender Tonintensitat abnimmt

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TISSUE LEVELS OF KANAMYCIN IN CORRELATION WITH OTO AND NEPHROTOXICITY

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stract The time courses of kanamycin (KM) levels in perilymph kidney liver brain and blood were observed by a bioassay method in normal guinea pigs and in mals pretreated with KM for 10 successive days In the lney and perilymph of the normal guinea pigs the in ted KM became highly concentrated and was elimi ted only after a protracted delay This tendency was ich more intensified in kidney and perilymph of the pre ated animals By contrast the KM in blood and brain is eliminated rapidly both in the normal and in the pre ated animals It is concluded that the high accumula n and slow elimination of KM by the inner ear and lney and moreover the enhancement of these phe mena by successive pretreatment are one of the impor nt factors in the origin of oto- and nephrotoxicity

he streptomyces antibiotics, such as streptoiycin and kanamycin (KM), are indispensable ven today for treatment of tuberculosis or seudomonas infections despite their oto- and ephrotoxicities To avoid these toxicities, it essential to understand their action mechnisms The studies of Vrabec et al (1965), oldrich (1965) and Stupp et al (1967) revealed iat the toxic streptomyces antibiotics became ighly concentrated in the cochlea and are eliinated from there only after a considerable eriod of time The genesis of the ototoxicity as been explained on the basis of these oints

However, the following questions still remain to be answered in order to explain the elective mechanism of their toxicities to the nner ear and the kidney

Are the accumulative propensity and delay limination of the drugs specific to the inner ar as opposed to other organs?

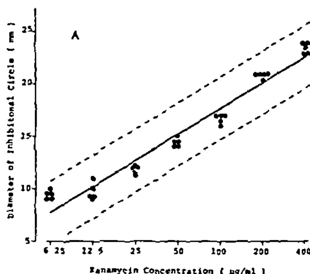
How does the repeated administration affect the processes of accumulation and elimination of these drugs in the various organs?

We investigated the time course of the concentration of the streptomyces antibiotics in the selected organs and blood before and after repeated administration We selected the cochlea and kidney to serve as the model for the vulnerable organs and the brain and liver for the non vulnerable organs We used kanamycin as test antibiotic, since no essential differences from other streptomyces antibiotics have as yet been reported for tissue concentration

METHOD

Sixty-six healthy albino guinea pigs weighing 300–400 g were divided into two equal groups at random The first group was the unpretreated control group The second group was pretreated with KM (400 mg/kg b w) for 10 successive days and sacrificed 48 hr after the last injection The animals of both groups were given KM (400 mg/kg b w) intramuscularly and sacrificed 1, 3, 6, 12 and 24 hr subsequently Several animals of both groups were sacrificed without KM injection, for control purposes

The chests of the animals were opened under ether anesthesia and the blood was aspirated from the hearts After decapitation the temporal bone, brain, liver and kidney were removed To collect perilymph from the opened bulla, a thin glass capillary was introduced through the oval and the round win



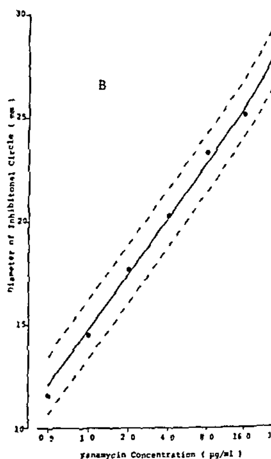


Fig 1 Standard curves of KM concentration in the function of the diameters of inhibitory circles of bacterial growth by known concentrations of standard KM solution in the cup method (*A*) and in the disc method (*B*) Each circle represents the mean values of 5 samples (*A*) or one value (*B*) — obtained by linear regression --- 95% confidence limits

ows into the scala tympani and the scala vestibuli, respectively The aspirated perilymph was examined under a microscope and any samples contaminated with blood were discarded

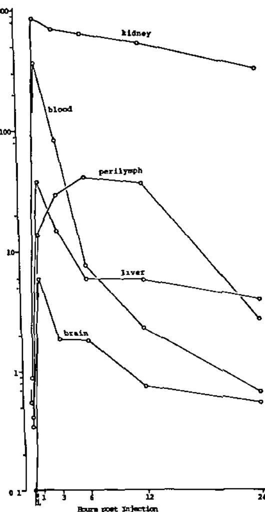
The brain, liver and kidney were homogenized and the homogenate or the whole blood was diluted with 0 1 mole phosphate buffer (pH 7 0) to the required antibiotic concentration

The agar diffusion method for the determination of KM was applied in the form of a cup test for blood and tissues and a disc test for perilymph A filter leaf No 50 (Toyo Roshi) with a diameter of 5 mm was used *Bacillus subtilis* ATCC 6633 served as test bacterium The minimum detectable range of KM was 0 01 μg/ml in the cup test and 0 5 μg/ml in the disc test The standard curves of the two methods are shown in Fig 1

RESULTS

Kanamycin levels in the normal animals

Fig 2 shows the time courses of KM lev in the various tissues The minimum value b fore KM injection represents certain non sp cific antibacterial activities, such as lysozy activity The maximum KM level in the tiss was highest in kidney (828±48) followed blood (361±29), perilymph (41±4), liver (3 5) and brain (6±1) Each value in parenthe represents the mean (microgram/ml or g w ±S E These maximum values were reach at 1 hr after injection—except that in the pe lymph, which appeared at 6 hr After 6 hr, t KM level in the blood, liver, and brain d creased to the low level of <10 μg/ml g w w However, the level in kidney and pe lymph remained high The KM level in liv blood, and brain showed a tendency tow



ig 2 The time courses of KM levels in the various tissues and fluids of the control group Each circle represents the mean value obtained from varying numbers of samples as indicated in Figs 3–8

rapid elimination, while that in perilymph and kidney showed a tendency to prolonged existence

Kanamycin levels in the pretreated animals

The KM levels in each tissue were compared between the control group and the KM pretreated group and evaluated statistically by the Student's *t* test

Figs 3 and 4 show the time course of the KM level in blood and brain, for both groups

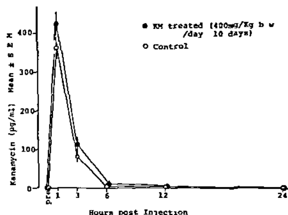


Fig 3 In Figs 3–8 the time courses of KM levels in the selected tissues and fluids are compared between the control group (O) and the pretreated group (●) These are the time courses of KM levels in the blood Each value represents the mean and S E obtained from 6 samples No significant difference in values was observed between the two groups at any time

No significant change was observed at any time in blood and brain The ratios of maximum KM levels in blood and brain of the pretreated group vis a vis those of the control group were 0 86 and 0 62 respectively And the ratios of KM levels in blood and brain at 24 hr in pretreated group vis a vis those in the control group were 0 41 and 0 62 respectively

In contrast, as shown in Figs 5, 6 7 and 8, the KM levels in perilymph of the scala vestibuli and the scala tympani in the kidney, and in the liver of those in the pretreated group were significantly higher than those of control group at the indicated times The ratios of maximum KM levels in the pretreated group vis a vis those in the control group were about 1 07, 1 64 1 83 and 2 43, respectively The ratios of KM levels at 24 hr in the pretreated group vis a vis those in the control group were about 5 71, 12 21, 4 34 and 8 67 in each tissue, respectively These ratios mean that KM accumulated more intensively in and was eliminated more slowly by those tissues of the pretreated group than by those of the control group Furthermore, the peak KM level in the perilymph, especially in that of the scala vesti

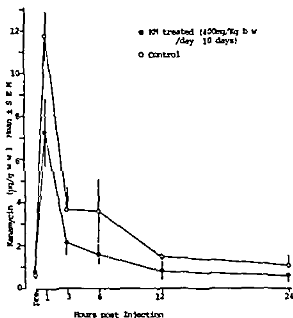


Fig 4 The time courses of KM levels in the brain Each value represents the mean and S E obtained from 6 samples No significant difference of the values was observed between the two groups at any time

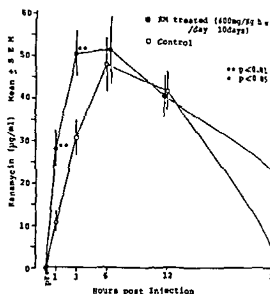


Fig 5 The time courses of KM levels in the scala t pani Each value represents the mean and S E obta from 6 to 12 samples The values of the pretreated mals are significantly higher than those of the co animals at the times and *p* values indicated by aster in Figs 5–8

buli, of the pretreated group occurred earlier that of the control group

DISCUSSION

The results show that the behavior of KM in the inner ear and the kidney is unique to the other organs The high KM concentrations in these tissues were accompanied by an increase in blood level but remained at a high level even after KM in blood had been almost eliminated Furthermore this tendency in the inner ear and the kidney, i e "high accumulation" and "slow elimination", were *enhanced* by repeated treatment of the same antibiotics, in contrast to the findings of Stupp (1970) Since these phenomena were not observed in blood and brain, they should correlate to the origin of oto and nephrotoxicity However the toxic mechanism of KM cannot be entirely explained by these phenomena alone, since the same kind of phenomenon even though not so remarkable, was also observed in the liver, which was believed not to be influenced by KM Some other cause of specific organ toxicity needs to be considered in order explain the specific vulnerability of the se sory hair cell in the cochlea and the urina tubules to KM (Tachibana, 1976) The abo mentioned specific behavior of KM in t

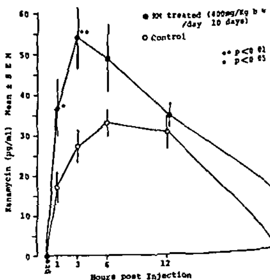
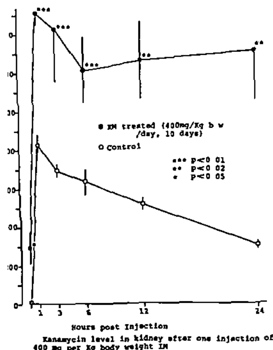


Fig 6 The time courses of KM levels in the scala ve tibuli Each value represents the mean and S E obtain from 6 to 12 samples



7 The time courses of KM levels in the kidney :h value represents the mean and S E obtained from imples

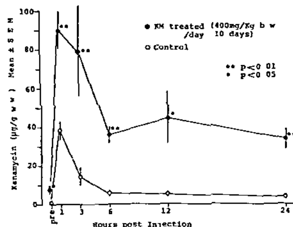


Fig 8 The time courses of KM levels in the liver Each value represents the mean and S E obtained from 6 samples

chlea and kidney, however, is considered be a factor of primary importance for the o and nephrotoxicities To understand the echanism in the cochlea we need to know the netration and elimination routes of the anti otics in this tissue Though the complete iswer to this question is not yet available, the ntative site of the penetration and elimina- on is, on morphological premises considered be the plexus cochlealis and the lateral wall the cochlea There is no evidence that the teral wall has any mechanism for the active imination of streptomyces antibiotics from ie perilymph However, Saito et al (1976) emonstrated that the lateral wall contained a reater amount of acidic glycosaminoglycan AGAG) than the other organs, and AGAG is elieved to bind streptomyces antibiotics and liminate them passively and slowly Further- nore Saito et al (1971) and one of the present uthors (Tachibana et al , 1976) revealed that he AGAG in the lateral wall decreased markedly after successive KM treatment There fore the *enhancement* of slow KM elimination from the perilymph by successive KM treatment may be caused by the diminished elimination capacity of AGAG in the lateral wall As for the plexus cochlearis Mootz et al (1972) reported on the morphological changes of this structure after KM treatment Thus, increased and faster accumulation of KM in the pretreated animals may be caused by the increased permeability of KM at this site

In conclusion it is plausible that the high accumulation and slow elimination of KM in certain organs and the enhancement of these phenomena by successive treatment are the salient factors in the origin of oto- and nephrotoxicity The mechanism of the varying susceptibility of each organ to streptomyces antibiotics still remains to be elucidated by modern sophisticated techniques

ZUSAMMENFASSUNG

Der Verlauf der Kanamyzin(KM)konzentrationen in der Perilymphe der Niere der Leber dem Gehirn und im Blut wurde durch eine biologische Methode bei normalen Meerschweinchen und bei den mit dauernden KM Injektionen während 10 Tage vorbehandelteten Meerschweinchen kalibriert In der Niere und der Perilymphe der normalen Tiere akkumulierte sich die gegebene KM hoch

gradig und wurde mit einer bedeutenden Verzögerung eliminiert Diese Tendenz wurde in der Niere und der *Perilymphe der vorbehandelten Tiere verstarkt Die KM* im Blut und Gehirn wurde sowohl bei normalen als auch bei vorbehandelten Tieren schnell ausströmen gelassen Es wird bemerkt, daß die hohe Konzentration der KM in der Perilymphe und der Niere und die spate Ausströmungstendenz und auch das Verstärken dieser Phenomena bei aufeinanderfolgender Vorbehandlung der Tiere sehr wichtig für die Ursache der Oto- und Nephrotoxizitāt der KM waren

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A POSSIBLE INVOLVEMENT OF ACIDIC GLYCOSAMINOGLYCANS IN KANAMYCIN OTOTOXICITY

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bstract The content of acidic glycosaminoglycans \GAG) was determined quantitatively by electrophoretic icroanalysis in the cochlea kidney and brain of the inea pig Kanamycin treatment (400 mg/kg body weight/ ay i m 10 successive days) reduced the content of GAG markedly in the lateral wall of the membranous ochlea Furthermore from the results of the ultrahisto- emical and freeze fracturing study we propose here an xcretion system for basic aminoglycoside antibiotics y means of the co-operation of spiral ligament cells and GAG produced by them Thus we have tentatively con uded both that kanamycin is excreted via the above entioned excretion system in the lateral wall of the embranous cochlea and that it damages this system to rm the *circulus vitiosus* of its ototoxicity

is well established that the membranous ochlea contains relatively high amounts of cidic glycosaminoglycans (AGAG, acid mu- opolysaccharides) (Saito et al , 1976) The xistence of AGAG in the tectorial membrane as attracted great interest in relation to the ossible role in the hearing process (Belanger, 953, Dohlman, 1960, Vilstrup et al , 1961, achibana et al , 1973, Yamamichi et al , 977) However, little is known as to the func onal role of AGAG in the lateral wall of the embranous cochlea (LWMC, stria vascularis spiral prominence + spiral ligament) which nows the highest value among the several tis ues in the cochlea (Saito et al , 1971) The resent study was designed to clarify one of e functional roles of AGAG in the LWMC relation to the mechanism of the ototoxicity f basic aminoglycosidic antibiotics

The improved, rapid quantitation of AGAG y electrophoretic separation, ultrahisto- hemical and freeze-fracture methods were used for the biochemical and morphological analyses of AGAG

METHODS

Throughout these experiments mature guinea pigs with normal Preyer reflex were used

(I) *Biochemical Analysis*

Animals were divided into two groups the normal control group and kanamycin intoxi- cated group Kanamycin intoxication was pro- duced by intramuscular injection of 400 mg of kanamycin sulfate (KM) per kg body weight daily for 10 days continuously On the day following the last injection, the animals were sacrificed, together with control group In some animals the threshold of the Preyer re- flex was checked daily The threshold before treatment was used as the zero level for each animal

More than forty cochleae, as well as several kidneys and brains were kept in 10% neutral formalin at 4°C for 2 weeks to 3 months The

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Abbreviations used

AGAG acidic glycosaminoglycans
LWMC, lateral wall of the membranous cochlea
KM kanamycin (sulfate)
PAM periodic acid methenamine silver
ChS A B,-C, chondroitin sulfate A B -C
HA hyaluronic acid
HS heparan sulfate
PAPS 3 phosphoadenosine 5 phosphosulfate

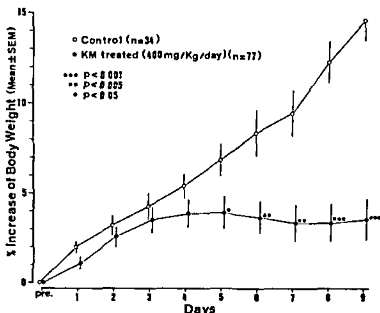
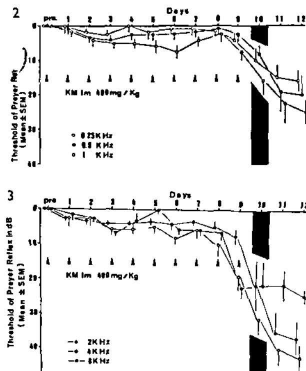
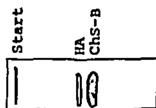


Fig 1 Relative changes of body The percentage increase in the kan (KM) treated group was significantl than that in control group after 5 in at the indicated *p* value Numbers animals are indicated in the parenth

lateral wall of the cochlea was then dissected and collected in the same solution The tissues were then cut into small pieces and transferred to absolute acetone for more than 3 days with several changes of acetone The samples dried in the desiccator for more than one and weighed using an electric balance (mal detectable change 0 5 μg) After dige



Figs 2 3 The shift in the Preyer reflex at the various frequencies Each value represents the mean of 10 animals One arrow indicates one injection of KM In the biochemical study animals were sacrificed after 10 injections (at the day indicated by solid column)



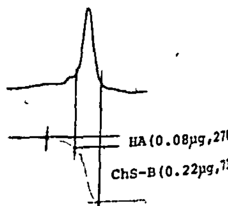


Fig 4 Electrophoretogram on cellulose acetate st the AGAG in the lateral wall of the membranous co (*top*) Densitometer tracing (*middle*) and reading th at 600 nm

ble I *Effect of kanamycin (KM) administration on the content of acidic glycosaminoglycans* *ÁAG)*

MC lateral wall of the membranous cochlea

	LWMC	Kidney	Brain
itrol	0 74±0 52 (3)	0 09±0 01 (8)	0 08±0 01 (4)
I treated	0 33+0 075 (3)*	0 08±0 01 (8)	0 07+0 01 (4)

<0 05 compared with control value by Student s *t* test
itents of AGAG are expressed as weight percent per dry weight Each value represents the mean + S E M ob- ied from the indicated number (in parentheses) of samples

protein by 5% pronase, AGAG was ex cted by ethanol precipitation in the pres ce of potassium acetate (Meyer et al , 1956 ito et al , 1970) The extracted AGAG were parated electrophoretically on a cellulose etate membrane in 0 2 M calcium acetate ffer and stained with 0 5% alcian blue in 3% etic acid The content of AGAG was deter ned by the scanning densitometer The pro- dure was essentially the same as reported eviously (Saito et al 1976)

(II) *Electronmicroscopic Analysis*

) *Ultrahistochemistry (periodic acid ethenamine silver (PAM) impregnation)*

he bony wall of the cochlea was opened and ced in 1% glutaraldehyde and 1% parafor aldehyde in 0 2 M phosphate buffer at pH 4 for 2 hr After washing in the buffer brief the specimens were refixed in 1% OsO_4 r 2 hr dehydrated in a graded series of ater–acetone mixture ending in absolute ace ine and embedded in Epon 812 according to e block surface technique The ultra thin ctions obtained were picked up on nylon ids and impregnated according to the meth d of Yajima (1971) Sections floated on the lver methenamine solution (5% methen mine 5% silver nitrate redistilled water % borax solution=10 1 9 1 in volume) for) min at 60°C After washing with redistilled ater specimens were floated on 0 25% gold lloride for 3 sec and again washed with re istilled water

(B) *Freeze fracturing*

The bony wall of the cochlea was opened in solution containing 2 5% glutaraldehyde and 0 1 M phosphate buffer (pH 7 4) and was left in the same fixative for about 4 hrs For freeze fracturing the specimens were equilibrated with 40% glycerol in saline, then frozen in liquid nitrogen Platinum carbon replicas were made as reported elsewhere by ourselves (Tachibana et al 1976 1976)

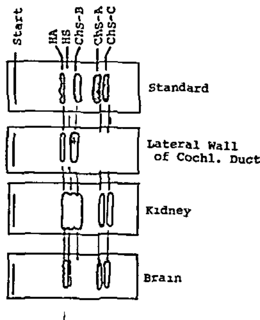
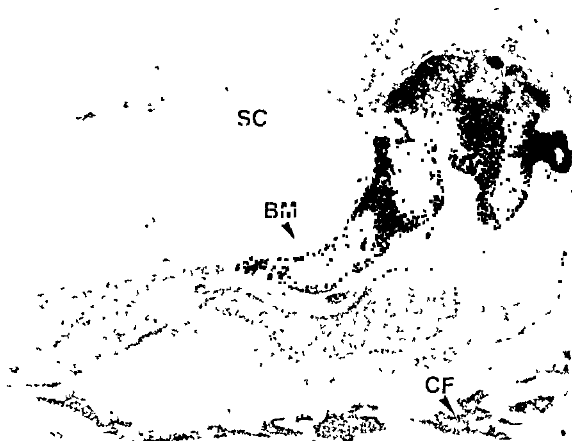


Fig 5 Electrophoretogram in the tested tissues The es *t mat on in the k dney* was extremely difficult by the tail ing of the spots Also heparan sulfate (*HS*) was not com mercially available as external reference and therefore HS content could not be estimated



Electronmicrograph of the capillary in the spiral ent Periodic acid methenamine silver (*PAM*) im egnation Note the impregnated basilar membrane (*BM*) and the collagen fibres (*CF*) and the amorphous sub surrounding them *SC* spiral ligament cell Scale ×15000

RESULTS

The percentage increase in body weight in the two groups is shown in Fig 1 The value in the KM group was significantly decreased compared with that in control group after 5 days' treatment

The threshold of Preyer reflex rose abruptly after about 10 injections of KM at all the frequencies examined (Figs 2, 3) In these figures, when the threshold exceeded the maximum sound pressure attainable, a value 5 dB greater than maximum attainable was used

(I) *Biochemical Analysis*

The LWMC (lateral wall of the membranous cochlea) contained ChS B (73%) > HA (27%) The kidney contained HA HS ChS B > ChS-C > ChS-A The brain contained ChS HA HS > ChS C The kinds and pattern not change after KM treatment in any t examined (Figs 4, 5)

The content of AGAG in each tissue dicated in Table I Both in the control a the KM treated groups, the LWMC sh the highest content of AGAG among th sues examined A significant difference observed only in the LWMC of the KM control groups by Student's *t*-test (Table

(II) *Electronmicroscopic Analysis*

(A) *PAM impregnation*

In the LWMC the basement membranes c capillaries were strongly impregnated, p ularly those of the spiral ligament (Fig The collagen fibres between the spiral



ɀ 7 Electronmicrograph of the lateral wall of the membranous cochlea PAM mp egnation Note the highly m gnated collagen f bres (*CF*) and the surround ng amorphous substance between the sp ral ligament cell (*SC*) *BC* basal cell of the vascular stria *Arrow* t ght junct on between them Scale 1 μm×7 800

ent cells and the amorphous substance sur und ng the fibres were also strongly impreg ated (Fig 6)

) Freeze fracturing

he many interest ng observations made with is technique will be reported in the near ture In relation to the purpose of this paper e authors would l ke to show here the large *umber of pinocytic vesicles on the cell mem* ane of the spiral ligament cells (Fig 7)

DISCUSSION

the LWMC (lateral wall of the membranous chlea) we found PAM impregnated material ainly in the basement membrane of the capil ry and collagen fibres and the ground substance surrounding them between the spiral l gament cells PAM impregnation is consid ered to demonstrate primarily the 1 ? glycol and/or alpha amino groups of glycoproteins and acidic residues of glycosaminoglycans (Rambourg et al 1967) PAM impregnated material in the LWMC is therefore—at least in part—AGAG Our ultrahistochemical data show that AGAG exist mainly in the spiral *ligament within the LWMC with close corre* spondence to the previous histochemical data at optical microscopic level (Musebeck et al 1964 Saito 1967) Considering the evidence that fibroblasts produce AGAG in tissue cul ture (Dorfman 1970) it seems most likely that AGAG in the LWMC are derived from the spiral ligament cells which are essentially fi broblasts

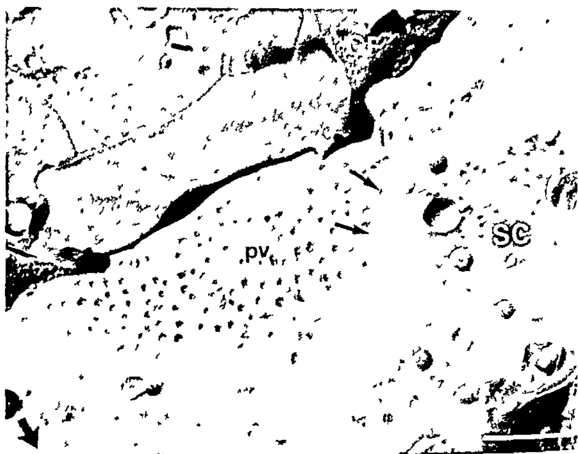


Fig 8 Electronmicrograph of the freeze fractured face of spiral ligament Note the large number of the pinocytic vesicles (pv) on the surface of the spiral ligament cell (SC) Also the cross surface of the pinocytotic vesicles (arrows) is seen CF collagen fibres Arrow in left corner indicates the direction of the platinum-carbon shadowing Scale 1 μm×32500

Also Higginbotham (1958) and Mora et al (1959) reported that AGAG or anionic derivatives of synthetic polyglucoses could form complexes with basic aminoglycosides and reduced their toxicities And the digestion of the drug–AGAG complexes by fibroblasts was suggested In this connection it is very interesting that spiral ligament cells showed pinocytosis (Fig 7) Therefore it is likely that the LWMC could have an excretion system for basic aminoglycoside antibiotics by means of co operation of spiral ligament cells and the AGAG produced from them

Our present data demonstrated that KM treatment at oto and nephrotoxic doses decreased the content of AGAG only in the LWMC in contrast to the other organs studied including the kidney Evidence that KM treatment damages the spiral ligament cells (I... al , 1973) and decreases the activity of P... synthesis enzyme (one of the key enzymes... AGAG synthesis) in the inner ear (Saito ... is also available These data demonstrate... the basic aminoglycosides themselves in... fere with the proposed excretion system... basic aminoglycosides in the LWMC in...

We also found the interesting phenome... (Tachibana 1977, Toyoda & Tachibana ... that KM penetrated more rapidly and rea... a higher value in the perilymph and excr... more gradually from it in the KM intoxica... animals than in the control animals

Taking all of the above data together... have tentatively concluded that the b... aminoglycosides were excreted to a great... gree via the excretion system for basic...

cosides in the LWMC and on the other , themselves damaged this system to form *rculus vitiosus* for their ototoxicities rther investigation by more sophisticated ods is necessary to elucidate the putative retion system for basic aminoglycosides" e inner ear The difference in mechanism een oto- and nephrotoxicity also remains answered The therapeutic usefulness of .G (ChS) against basic aminoglycoside ıxicity is already reported (Shida, 1957) should be re evaluated

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ZUSAMMENFASSUNG

ıantitativen und qualitativen Analysen der sauren osaminoglykane (SGAG) in der Schnecke den n und im Gehirn von Meerschweinchen wurden mit lektrophoretischen Mikroanalysenmethoden durch ırt Nach Kanamycinbehandlung (taglich 400 mg pro orpergewicht i m 10 Tage) verringerte bedeutend Gehalt von SGAG der lateralen Wand der hautigen ecke Von unseren Untersuchungsergebnissen mit Ultrahistochemie und Gefrierfrakturtechnik wurde ein Exkretionssystem für basische Aminoglykosi vorgeschlagen unter Mitwirkung der Spiralliga zellen und der von ihnen produzierten SGAG Die ichkeit daß Kanamycin von diesem Exkretions- m abgesondert wird und es deshalb beschadigen te sowie die folgende Ototoxizitat in Form eines *ulus vitiosus* wurde diskutiert

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ION TRANSPORT IN GUINEA PIG COCHLEA

I *Potassium and Sodium Transport*

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Abstract The scala vestibuli and/or tympani in anesthetized guinea pigs was perfused with artificial perilymph containing ^{42}K and ^{22}Na for periods ranging from 5 to 60 minutes Sound evoked responses were recorded during the perfusion The activities of ^{42}K and ^{22}Na in perilymph and endolymph were determined with a germanium detector in conjunction with a multichannel analyser The perfusion of scala vestibuli and/or tympani did not result in appreciable changes in electrical responses The introduction of ^{42}K and ^{22}Na into the scala tympani resulted in the rapid appearance of these radioisotopes in perilymph of the scala vestibuli The activities of ^{42}K and ^{22}Na in perilymph of the scala tympani remained low with perfusion of scala vestibuli This is in agreement with our findings that the clearance of these ioisotopes was faster in scala tympani than in scala buli Our results further indicate that the endolymph up ^{42}K and extruded ^{22}Na against the concentration ients The transport of ^{42}K and ^{22}Na across the coch ar partition was inhibited by anoxia or local application of ouabain Uptake of ^{42}K in the endolymph from perilymph of the scala vestibuli was comparable to uptake from perilymph of the scala tympani These results suggest that $^{+}$K is actively transported from perilymph to endolymph and $^{+}$Na is extruded from endolymph to perilymph and that the active transport of $^{+}$K takes place in the stria vascularis By adopting a two-compartment system the transport rate constant of $^{+}$K was computed from our experimental data

Numerous experiments have shown that the function of the cochlear hair cells is intimately dependent on the unique ion concentration of the endolymph, which is characterized by high potassium and low sodium concentration (Konishi et al, 1966, Konishi & Kelsey, 1968, 1973, Kuijpers, 1969) In addition the polarization of the endolymph plays an essential role enabling the hair cells to develop a receptor potential in response to minute acoustic stimuli The maintenance of the ion concentration in the endolymph depends upon the struc and functional integrity of the plasma m brane of those cells which serve as the ba between perilymph and endolymph I likely that ototoxic agents can cause de tion of the endolymph–perilymph barrier consequently lead to a disturbance of mechano-electrical transduction mechanis the cochlea

The permeability of the endolymph-p lymph barrier to various ions can be de mined by use of radioisotopes The concen tion of the radioisotopes needed is as a rul smaller than that of the normally existing Therefore one can safely assume that physicochemical state of the barrier is no tered by the radioisotope tracer By using radiotracer technique in the cochlea of gu pig, Rauch et al (1963) argued that Reissn membrane is a site of active transport of and $^{+}$Na Their work made a significant co bution to understanding the function of Re ner's membrane but their results seem to s their endolymph samples to be contamin with the perilymph

This report is the first of a series on p posed experiments using radioisotope tr methods in an effort to examine the poten alteration of the endolymph–perilymph bar caused by various ototoxic agents

This first paper deals principally with transport of $^{+}$K and $^{+}$Na through the coch

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ition under the condition of continuous 'usion in the cochlea of guinea pigs Our ilts demonstrate that ^{+}K is actively trans- :ed into the endolymph against an electro- mical gradient and that ^{+}Na is prevented n entering the endolymph On the basis of results, we suggest that the stria vascularis he principal contributor to active cation isport in the cochlea

ι preliminary report on this work was pre ted at the 92nd meeting of the Acoustical :iety of America (Konishi et al , 1976)

METHODS

ilthy pigmented guinea pigs (NIH strain) sthetized with pentobarbital sodium were d The cochlear potentials in response to iustic stimuli were recorded from the basal n of the cochlea with differential electrodes isaki et al , 1952) The techniques for re ·ding the cochlear potentials, including :hlear microphonics (CM), summating po itial (SP) whole nerve action potential of : auditory nerve (AP), and endocochlear po- itial (EP) have been fully described in our :vious paper (Konishi et al , 1961) Special re was taken to minimize fluid leakage from : holes prepared for the intracochlear elec ides In most cases the AP in response to ch of eight successive acoustic stimuli was eraged by a computer The tone bursts used

ectively

The perfusion technique employed was sim ir to that described in our previous paper ,onishi & Kelsey, 1968) When both scala :stibuli and tympani were perfused, a perfu on pipette whose tip was approx 100 μm in ameter, was inserted into the scala tympani the basal turn of the cochlea and a hole to rve as an outlet was made in the scala vesti- ıli of the basal turn When the scala vestibuli or scala tympani was perfused separately, a perfusion pipette was placed in the corresponding scala of the basal turn and an outlet was made at the apex The perfusion rate was approximately 2 μl/min and the period of perfusion ranged from 5 to 60 min

^{42}K (12 7 hours half-life) of high specific activity and ^{22}Na (2 6 years half life) were obtained in chloride form from New England Nuclear, Boston, Massachusetts These original radioactive solutions were mixed with modified Ringer's solution so that the per fusate had the following composition (mM), NaCl 137, KCl 5, $CaCl_2$ 2, NaH_2PO_4 1, $MgCl_2$ 1, $NaHCO_3$ 12, glucose 11 As a rule the activity of ^{22}Na in the perfusate was 125 nCi/μl and that of ^{42}K ranged from 250 to 300 nCi/μl at the time of preparation The perfusate was kept at room temperature (18°C)

The technique of sample collection of the endolymph has been described previously (Mandelsohn & Konishi, 1969) and therefore only a brief description will be given here A double-barrelled micropipette was prepared whose tip was approx 5–10 μm in diameter The tip of one barrel used to collect the endolymph was then filled with water equilibrated mineral oil The other barrel was filled with Ringer's and used as an EP recording electrode The double barrelled pipette, mounted on a micromanipulator, was inserted into the scala media of the basal turn through the spiral ligament About 1 μl of fluid was collected by gentle suction when the potential recording barrel registered stable EP, indicating the tip to be in the scala media The mineral oil prevented fluid from entering the barrel until suc tion was applied When the endolymph was collected smoothly, EP did not show any sig nificant change When EP showed a sudden drop or decreased continuously by more than 10 mV, the sample was discarded

Samples of perilymph were taken from the scala vestibuli and scala tympani of the basal turn of the cochlea A single glass micropipette was used whose tip was ca 100 μm in diame ter The shank was coated with lacquer so as

to form a small bead at about 100 μm from the tip The tip was then filled with water-equilibrated mineral oil After the tips of the differential electrodes were temporarily lifted from the cochlea, the collection micropipette was inserted into either scala vestibuli or tympani About 1 μl of perilymph was obtained by applying gentle suction The whole procedure of collection of endolymph and perilymph required 3 to 4 min Samples were taken at times ranging from 5 to 60 min after the start of perfusion Separate animals were used for each time, since it is difficult to collect more than one sample of the endolymph and perilymph in the scala vestibuli and tympani An exception to the above was made in one set of experiments in which the radioactivity in the perilymph was determined as a function of time with the same animal

The activity of ^{42}K and ^{22}Na was determined using a solid state germanium detector in conjunction with a multichannel analyser The system was calibrated with known quantities ^{42}K and ^{22}Na by determining the total num- 'of counts in selected channels for a live of 1 000 sec This time was sufficient t reduce the standard deviation in the sample from counting errors to less than 5% of the total counts Activity determinations were corrected for the decay of ^{42}K The samples containing ^{42}K and ^{22}Na were transferred into 10 μl calibrated glass pipettes Both ends of the samples were sealed with water-equilibrated mineral oil The volumes of samples were calculated by measuring their length under a microscope Each sample in a 10 μl calibrated pipette was carefully centred under the detector crystal in a specially constructed holder Under the condition used small changes in the position of the sample did not cause detectable changes in the geometrical efficiency Thus the method used allowed us to express the activities of ^{42}K and ^{22}Na in terms of nCi/μl

Special methods and procedures used in specific experiments will be described in the Results section

RESULTS

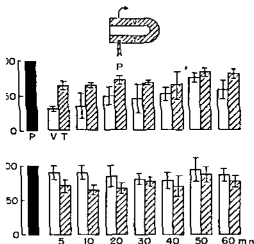
The results reported here were based on obtained from 110 guinea pigs

1 *Cochlear potentials during perfusion*

The average magnitude of CM in respon 6 kHz tone bursts at 70 dB SPL was 45 when the CM was recorded from the t turn The CM was stable during perf of the scala vestibuli and tympani when was taken to remove the accumulated from the auditory bulla In a few cases was slightly increased in magnitude by a f of 10% during the first few minutes of p sion There was a slight decrement of CM ing perfusion, ranging from 5 to 20 minute cases of a period of perfusion longer tha minutes, the CM showed a gradual decrea magnitude but the decrease of CM did exceed more than 30% of the initial CM W the perfusion was carried out in the scala v buli or tympani separately, the suppressi CM was less in both cases than when the tire perilymphatic space was perfused

The magnitude of AP to 6 kHz tone b at 70 dB SPL showed a larger variation that of the CM It ranged from 250 to 10 with a mean value of 180 μV The AP was porarily suppressed during the first 2–5 utes of the perfusion The decrease in ranged from 10 to 50% of the original The AP generally showed full recovery minutes after the perfusion was initiated was stable during the rest of the perfu period When the scala tympani was perf separately, the temporal decrease in AP a ciated with initiation of perfusion was sim to that observed during the perfusion of entire perilymphatic space The decreas AP was much less in the perfusion of the s vestibuli than in that of the scala tympani

Changes in SP were noted during the few minutes of perfusion When the per ate was introduced into the scala vestib the negative SP was increased in magnitu The perfusion of the scala tympani tended



/ Mean activities of ⁴K (upper panel) and ²²Na :r panel) in samples obtained from scala vestibuli) and from scala tympani *T* (▨) at various durations :rfusion indicated below the bars The perilymphatic e was perfused as shown in the top diagram The acti s in samples are normalized by taking those in the per :e *P* (■) as 100% Four to six animals were used for perfusion time except for the 5 min perfusion in h only ⁷ animals were used Vertical range bars in :e standard deviations

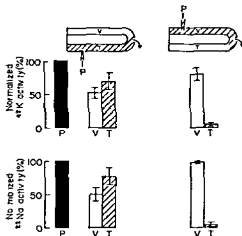


Fig 2 Mean activities of ⁴²K (upper panel) and ²²Na (lower panel) under two different perfusion conditions as shown in the top diagrams The duration of perfusion was 20 min Activities in perilymph of scala vestibuli *V* (□) and scala tympani *T* (▨) are normalized by taking those in perfusate *P* (■) as 100% Six animals were used for each condition Vertical range bars indicate standard deviations

ke the SP less negative and sometimes a itive SP was observed However, such nges in SP gradually disappeared 10 min :r perfusion was initiated and the SP was ole during the rest of the perfusion period

n the preparations which were used for de mination of activity in the cochlear fluids EP was not recorded continuously during fusion but was measured at the time of col tion of the endolymph The average value the EP was 82 5 mV which was comparable that obtained from non perfused cochlea During collection of the endolymph the EP l not show a decrease of more than 10 mV ıe CM and especially the AP were suppres d but decreases in these responses were less in 20% when the endolymph was collected

the perilymph were taken within 10 sec ıds The decrease in the CM and AP ranged om 20 to 50% However, in most cases the CM and AP showed partial recovery after col lection was completed

2 *Concentration of ⁴²K and ²²Na in perilymph*

When the perfusate containing ⁴²K and ²²Na was introduced into the scala tympani of the basal turn and both scala vestibuli and tympani were perfused the concentration of ⁴²K and ²²Na in samples of the perilymph taken from the scala tympani ranged from 65 to 85% of those in the perfusate and remained almost unchanged as the period of perfusion was prolonged (Fig 1) ⁴²K and ²²Na in samples from the perilymph of the scala vestibuli were de tectable 5 minutes after perfusion was initiat ed As the period of perfusion was prolonged, the concentration of ²²Na in the perilymph of the scala vestibuli did not increase but it was always higher than that in the perilymph taken from the scala tympani On the other hand, the concentration of ⁴²K in the perilymph of the scala vestibuli had a tendency to increase as the period of perfusion increased and it was noted that samples from the scala vestibuli in-

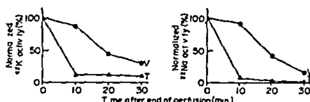


Fig 3 One example of clearance of ^{42}K (left panel) and ^{22}Na (right panel) after the end of perfusion of perilymphatic space ● Activity in perilymph of scala vestibuli V, ▲ activity in perilymph of scala tympani T The activities are normalized by expressing those obtained at and of perfusion as 100% *Abscissa* Time elapsed after perfusion Three animals were tested and 2 other animals showed similar clearance

dicated a substantially lower concentration of ^{42}K than those obtained from the scala tympani

It is seen in Fig 2 that when the scala tympani alone was perfused for a period of 20 minutes, concentrations of ^{22}Na and ^{42}K in samples taken from the scala vestibuli were about 30% lower than those obtained from the scala tympani Concentrations of ^{42}K and ^{22}Na in the lymph of the scala vestibuli after perfusion scala vestibuli alone showed 70 to 90% 90 to 100% respectively of those in the erfusate Surprisingly the activity of ^{22}Na and ^{42}K in samples taken from the scala tympani showed only a few percent of the activity in the perfusate

We examined the activity of ^{22}Na and ^{42}K in the perilymph of both scala vestibuli and tympani when the scala vestibuli was perfused for a period of 20 min in animals in which the cochlear aqueduct had been surgically obstructed Obstruction of the cochlear aqueduct was performed by drilling with a fine burr across the cochlear aqueduct A minute amount of bone wax was applied to obstruct the cochlear aqueduct In those animals CM and AP were not suppressed after the obstruction The activities of ^{22}Na and ^{42}K in samples obtained from the scala tympani increased by a factor of 2 to 3 but remained lower than those in samples from the scala vestibuli

The activities of ^{22}Na and ^{42}K in samples taken from the perilymphatic space decreased rapidly when the perfusion was discont... Fig 3 shows clearance of ^{22}Na and ^{42}K a... the end of perfusion of the scala vestibuli... tympani in one animal in which samples of perilymph were periodically taken The decline in activity was much faster in sam... obtained from the scala tympani than in th... from the scala vestibuli Thirty minutes af... the end of perfusion the loss of activity of... was about 80% in the perilymph of the vestibuli, whereas ^{22}Na was scarcely dete... able in the perilymph of the scala tymp... There was no noticeable difference in the r... of clearance between ^{22}Na and ^{42}K

3 *Concentration of ^{42}K and ^{22}Na in endolymph*

1 *Normal guinea pigs* It is noted in Fi... that when the scala vestibuli and tympani w... perfused, the ^{42}K concentration in the en... lymph exceeded that in the perilymph in... scala tympani within 5 min after perfusion...

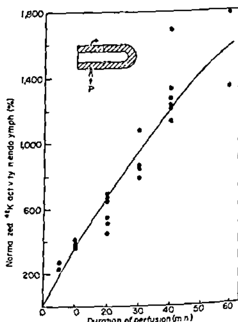
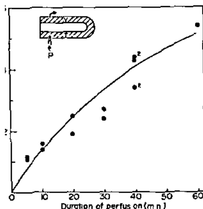


Fig 4 Uptake of ^{42}K in endolymph as a function of ... fusion duration The scala vestibuli V and tympani ... were perfused as shown in inset diagram at top Acti... in endolymph is normalized by taking that obtained i... as 100% ● Data points The line is the least square... fit to the points (see text)



5 Uptake of ^{22}Na in the endolymph as a function erfusion duration See caption to Fig 4

nmenced As perfusion continued to 40 1, the concentration of ^{42}K rose to about 12 es the concentration of ^{42}K in samples en from the scala tympani As shown in ; 5, the concentration of ^{22}Na in the endonph was less than 1% of that in samples obned from the scala tympani Even when per ion was prolonged by 60 minutes, the ^{22}Na ncentration in the endolymph did not reach 5% of that found in the perilymph from the ala tympani

As noted above, in the perfusion of the scala mpani alone, the activities of ^{42}K and ^{22}Na in e scala vestibuli were comparable to those in e scala tympani When the site of perfusion is switched from scala tympani to scala ves buli their activities in the scala tympani were ss than those in the scala vestibuli However e activities of ^{42}K and ^{22}Na in the endo mph did not show any marked differences ider these two conditions, when their activi es were normalized by expressing those obined from the perilymphatic space into hich the perfusate was introduced as 100%

These findings were further substantiated y a dual perfusion The simultaneous perfu ons were carried out with 'hot' solution ontaining ^{42}K and ^{22}Na and "cold' solution ithout ^{42}K and ^{22}Na Three experimental procedures were employed Fig 6 shows the activities of ^{42}K and ^{22}Na in the perilymph and endolymph under these conditions of perfusion for a period of 20 min In the first procedure "hot" solution was introduced into both scala vestibuli and tympani (left panel in Fig 6) In the second procedure the "hot" solution was introduced into the scala tympani and "cold' solution was simultaneously introduced into the scala vestibuli (middle panel) In the third procedure a similar perfusion was carried out in the reverse manner, "hot" perfusion of scala vestibuli and "cold' perfusion of scala tympani The electric responses during the dual perfusion did not show differences from those observed in the perfusion of the scala tympani The activities of ^{42}K and ^{22}Na were, of course, very weak in the samples taken from the scala which was perfused with the "cold' solution On the other hand, their activities in the samples taken from the scala into which the "hot" perfusate was introduced, were comparable to those found in the "hot' perfusate The activities of ^{42}K and ^{22}Na in the

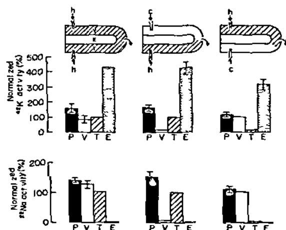


Fig 6 Mean uptake of ^{42}K (upper panel) and ^{22}Na (lower panel) in endolymph *E* and perilymph of scala vestibuli *V* and scala tympani *T* under three different perfusion conditions as shown in diagrams at top left perfusion of scala vestibuli and tympani with hot perfusate *h* mid dle simultaneous perfusion of scala tympani with hot" perfusate and perfusion of scala vestibuli with cold per fusate *c* right simultaneous perfusion of scala tympani with cold" perfusate and perfusion of scala vestibuli with hot perfusate Duration of perfusion was 20 minutes Activities are normalized to those of *V* or *T* Four animals were used for each condition Vertical range bars indicate standard deviations

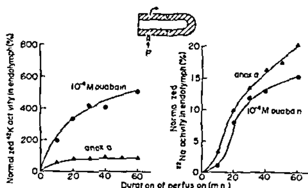


Fig 7 Effects of anoxia (▲) and 10^{-4} M ouabain (●) on uptake of ^{42}K (left panel) and ^{22}Na (right panel) in endolymph Scala vestibuli and tympani were perfused as shown in top diagram *Ordinate* Activity in endolymph normalized to that in perilymph of the scala tympani Two to three animals were used to obtain each data point

endolymph showed little difference under these three perfusion conditions

2 *Effect of ouabain* The effect of ouabain on the uptake of ^{42}K and ^{22}Na in the endo[lymph] was examined by adding ouabain to the [perfu]sate The concentration of ouabain was [raised] to 10^{-4} M Introduction of ouabain into [the] perilymphatic space resulted in the suppression of cochlear potentials to acoustic stimuli The decline of the electric responses we observed was similar to that reported previously (Konishi & Mendelsohn, 1970) As demonstrated in Fig 7, the concentration of ^{42}K in the endolymph was diminished by 10^{-4} M of ouabain in the perfusate This suppressive effect was somewhat greater when the concentration of ouabain was increased to 10^{-3} M

The concentration of ^{22}Na in the endolymph was increased by local application of ouabain (Fig 7) Compared with that found in normal animals (Fig 5), ^{22}Na concentration increased slightly 10 min after the start of perfusion and as the perfusion continued the rate of increase of ^{22}Na concentration increased With 10^{-3} M of ouabain the ^{22}Na concentration in the endolymph was greater than that obtained with 10^{-4} M

3 *Effect of anoxia* The cochlear responses to acoustic stimuli showed a rapid decli[ne after] the intravenous injection of a lethal [dose of] pentobarbital sodium The perfusion o[f scala] vestibuli and tympani was carried out [immediately] after the injection The concentration [of ^{42}K] in the endolymph did not reach the leve[l of the] concentration in the scala tympani (Fig[7) in] contrast to that shown in Figure 4 Th[e con]centration of ^{22}Na in the endolymph as [shown] in Figure 7, increased in the post[mortem] state As the duration of perfusion inc[reased] the activity of ^{22}Na increased marked[ly and] after 60 min of perfusion reached 20% [of the] activity in the perilymph of the scala t[ympani]

4 *Effect of perfusion* In order to as[certain] that the perfusion did not alter the perm[eabili]ty of the cochlear partition to $^{+}$K or $^{+}$N[a], perfusion of the perilymphatic space wa[s per]formed with ' cold ' perfusate for a [period] ranging from 20 to 60 min Immediatel[y after] the initial perfusion, perfusate contain[ing ^{42}K] and ^{22}Na was again introduced into th[e peri]lymphatic space The activities of ^{42}[K and] ^{22}Na in the endolymph did not show a[ny dif]ferences from those obtained withou[t] "cold perfusion which were described [in the] previous section

4 *Quantitative aspects of $^{+}$K and $^{+}$Na transport across the cochlear partition*

In order to simplify the treatment [of the] transport process occurring in the guin[ea pig] cochlea, assume that the cochlea can [be re]presented by two compartments Let the [inner] compartment represent the scala media with the endolymph and the outer com[part]ment, the perilymphatic space filled wi[th] perilymph Let J_{in} (mmole/min) denot[e the] flux of given nonradioactive ions fro[m the] outer compartment to the inner compar[tment] and their flux in the opposite directi[on] J_{out} The corresponding flux of radio[active] ions may be denoted by $^{*}J_{in}$ and $^{*}J_{out}$ [Under] perfusion conditions, we may assume th[at the] concentration of ions in the perilymph re[mains] constant For this condition the two

t system in effect becomes a one compart-
:t system

he unidirectional fluxes of radioactive
; can be expressed by

$$=\frac{*C_{out}}{C_{out}}\,J_{in} \text{ and } *J_{out}=\frac{*C_{in}}{C_{in}}\,J_{out} \qquad (1)$$

:re C_{out} and C_{in} are concentrations of non-
ioactive ions in the outer and inner com-
tment respectively, and $*C_{out}$ and $*C_{in}$ are
icentrations of radioactive ions in the outer
l inner compartment respectively In the
ady state, the net flux of nonradioactive
s is zero Thus

$$_{t}=J_{in}=J \qquad (2)$$

om eqs (1) and (2)

$$_{in}=\frac{*C_{out}}{C_{out}}\,J$$

$$_{out}=\frac{*C_{in}}{C_{in}}\,J \qquad (3)$$

ie rate of change in the concentration of ra-
oactive ions in the inner compartment can be
:pressed by

$$_{in}\,\frac{d*C_{in}}{dt}=*J_{in}-*J_{out} \qquad (4)$$

here V_{in} is the volume of the inner compart-
ent

From eqs (3) and (4)

$$_{in}\,\frac{d*C_{in}}{dt}+J\,\frac{*C_{in}}{C_{in}}=J\,\frac{*C_{out}}{C_{out}} \qquad (5)$$

nce the perilymphatic space is being per-
sed, $*C_{out}$ is assumed to be constant

By solving eq (5)

$$C_{in}\exp[Jt/V_{in}C_{in}]=\frac{C_{in}}{C_{out}}*C_{out}\exp$$

$$Tt/V_{in}C_{in}]+\text{const} \qquad (6)$$

'hen $t=0$, $*C_{in}=0$

'hus $\text{Const}=\frac{-C_{in}*C_{out}}{C_{out}}$ (7)

From eqs (6) and (7),

$$\frac{*C_{in}}{*C_{out}}=\frac{C_{in}}{C_{out}}(1-\exp[-Jt/V_{in}C_{in}])$$

or

$$\ln\left(\frac{C_{in}}{C_{out}}-\frac{*C_{in}}{*C_{out}}\right)=\ln\left(\frac{C_{in}}{C_{out}}\right)-\frac{J}{V_{in}C_{in}}\,t \qquad (8)$$

From eq (8) the transport rate constant, $J/V_{in}C_{in}$ can be calculated, if C_{in}, C_{out}, $*C_{in}$ and $*C_{out}$ are known

The concentration of ^{+}K in the endolymph reported by various investigators (in guinea pigs Johnstone et al , 1963, Konishi & Kelsey, 1973, in rats Bosher et al , 1973) ranged from 130 to 190 mM Assuming that C_{in} of ^{+}K is 150 mM and C_{out} of ^{+}K is 5 mM, Fig 8 shows the relationship between ln $(C_{in}/C_{out}-*C_{in}/*C_{out})$ and t, perfusion time, expressed by eq (8) By utilizing the least square best fit, the regres sion line can be expressed by

$$\ln\left(\frac{C_{in}}{C_{out}}-\frac{*C_{in}}{*C_{out}}\right)=3\ 40-0\ 0126t \qquad (9)$$

Using an analysis of variance technique (Dixon & Massey, 1957), the data were tested for line arity and found not to differ significantly ($P=0$ 05) from the above line The transport rate constant for ^{+}K was 0 0126 min^{-1} and the half-time for exchange was 55 1 min

The concentration of ^{+}Na in the endolymph determined by various investigators (John stone et al , 1963, Konishi & Kelsey, 1973, Bosher et al , 1973) shows considerable varia tions and ranges from 2 5 to 0 5 mM Assum- ing that the concentration of ^{+}Na is 1 mM in the endolymph and 140 mM in the perilymph, the transport rate constant was 0 021 min^{-1} and the half time exchange was 32 6 minutes If the concentration of ^{+}Na is 2 mM in the endolymph, the transport rate constant be- comes 0 007 min^{-1} and the half time exchange 94 8 min Since the flux (J) is the product of the volume, concentration and rate constant, we may determine the ratio of the flux of ^{+}K to that of ^{+}Na When the ^{+}Na concentration is assumed to be 1 mM or 2 mM, the flux ratios are 90 and 135 respectively

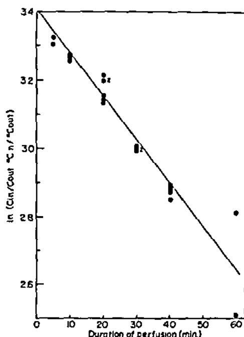


Fig 8 ln $\{(C_{in}/C_{out})-(^*C_{in}/^*C_{out})\}$ as a function of duration of perfusion C_{in} and C_{out} represent nonradioactive K concentration in endolymph and perilymph (assumed to be 150 mM and 5 mM respectively) $^*C_{in}$ and $^*C_{out}$ represent radioactive K concentration in endolymph and perilymph respectively The regression line obtained by squares best fit has a slope of 0 013 an intercept of correlation coefficient is 0 92

DISCUSSION

Since Smith et al (1954) demonstrated that the endolymph of guinea pig has a very high concentration of $^+$K and a very low concentration of $^+$Na, extensive studies of the distribution of $^+$K and $^+$Na in the cochlear fluids have been carried out in various species (Katsuki, 1965) and under various pathological conditions (Mendelsohn & Katzenberg, 1972, Bosher et al , 1973, Cohen et al , 1971) The movement of $^+$K and $^+$Na across the cochlear partition is very important to revealing the basic mechanism of cochlear function and yet only a few studies have been reported (Rauch, et al , 1963, Choo & Tabowitz, 1964, 1965) In reviewing their reports critically, their results and interpretations are inconsistent and conflicting It is likely that these conflicting results can be attributed to *technical difficulties in the collection* of endolymph without contamination by the perilymph and secondly to a lack o quate monitoring of the cochlear fl ing the introduction of radioisotopes in cochlear fluids In out experiments evoked responses were periodically during the perfusion and the EP was moni during collection of the endolymph an endolymph collected was discarded if th decreased its magnitude more than 10 m ing collection The determination of $^+$K $^+$Na in the endolymph samples collected b method (Konishi & Kelsey, 1973) agreement with the report by Bosher (1973) or Johnstone et al (1963) Th is reasonable to assume that contaminati the perilymph is negligible in our experim

In addition there is a good possibility chemical injuries of the cochlear partitio sociated with the intracochlear injectio count for the conflicting results reported viously (Rauch et al , 1963) It has been sh that the electrical responses of the cochle *modified by either alteration of the electr* concentration of the perilymph (Konish Kelsey, 1968, 1970, 1973) or changes in hy static pressure in the cochlear fluids (Tasa al , 1954) Therefore these changes may c modification in the permeability of the c lear partition to electrolytes For example isotonic KCl solutions used by Rauch e (1963) may have resulted in chemical injur cells of Reissner's membrane Our ex ments show that the sound evoked respo did not change substantially during the pe sion of the perilymphatic space It is lik therefore, that any changes in hydros pressure caused by perfusion and replacem of perilymph with the perfusate used by us not of sufficient magnitude to disturb cochlear responses This conclusion was ther substantiated by the fact that uptake ^{42}K and ^{22}Na in the endolymph was not m fied by prior perfusion of the perilymph space with "cold' solution

The activities of ^{42}K and ^{22}Na in the p lymph of the scala vestibuli of the basal t are detectable within 5 min after the f

te is introduced into the scala tympani of basal turn (Fig 1) The presence of the ity indicates that the perfusate reaches scala vestibuli not only through the helico a (longitudinal pathway), but also through radial pathway It is unlikely that ^{42}K and introduced into the scala tympani diffuse the scala media and then reach the scala ibuli, as Jahnke (1975) reported that zon occludens of non sensory epithelia seal cochlear duct Thus the spiral ligament es principally as a radial pathway, in ement with a previous report in which ndorf et al (1962) observed the rapid fluid hange in the spiral ligament by using the l staining technique Rauch et al (1963) in ed isotonic ^{42}K or $^{24}NaCl$ solution into the la vestibuli and measured uptake of ^{42}K and a in the endolymph in order to determine ransport occurs across Reissner s membrane They interpreted their results as show that radioisotopes in the endolymph are nsported across Reissner s membrane wever, our findings indicate that ^{42}KCl or aCl solution injected into the scala vestibuli easily diffuse into the spiral ligament and refore do not support their interpretation

One puzzling result is the fact that perfusion he scala vestibuli with radioactive solution ults in low activities in perilymph of the la tympani and it occurs consistently re dless of the duration of perfusion This re t can be explained by the rapid clearance of ioisotopes in the scala tympani As shown ig 3 the clearance of ^{42}K and ^{22}Na is much ter in the scala tympani than in the scala tibuli The perfusate introduced into the la vestibuli reaches the scala tympani ough the spiral ligament but the clearance ^{42}K and ^{22}Na is so fast in the scala tympani t an increase in concentration of these ra isotopes cannot be observed This may ac nt for the fact that an increase in K concen tion in the perilymph of the scala tympani is re effective in suppressing the CM and AP in that of the scala vestibuli (Tasaki & Fernndez 1952) Another possible explanation is that as the scala tympani communicates the subarachnoidal space through the cochlear aqueduct, opening of an outlet in the scala tympani may create leakage of the cerebrospinal fluid into the scala tympani and, as a consequence, the concentration of these radio isotopes in the scala tympani is diluted with the cerebrospinal fluid This possibility can not be a major factor as obstruction of the cochlear aqueduct did not substantially increase the activities of ^{42}K and ^{22}Na in the scala tympani Kimura & Perlman (1956) re ported that profound changes in the inner ear are produced only when the inferior cochlear vein is obstructed at the level at which some of the collaterals are also obstructed From the fact that the surgical blocking of the cochlear aqueduct did not suppress the electrical re sponses of the cochlea, it is apparent that the surgical procedure used to obstruct the cochlear aqueduct did not interfere with the venous drainage of the cochlea

We have consistently found that, when the perilymphatic space was perfused with arti ficial perilymph containing ^{42}K, the endolymph took up ^{42}K very quickly and within 5 min after perfusion, the ^{42}K concentration in the endolymph was greater than in the perilymph (Fig 4) On the other hand, the uptake of ^{22}Na in the endolymph was extremely small (Fig 5) Although our experimental conditions are different from those used by Rauch et al (1963), our results are qualitatively in agree ment with their findings Because the endolymph is a rich ^+K and low ^+Na medium and positively polarized with respect to the peri lymph, the transport of ^+K ions is against the electrochemical gradients Also the transport of ^{42}K and ^{22}Na was inhibited by anoxia and ouabain as shown in Fig 7 Ouabain generally inhibits active transport (Caldwell & Keynes, 1959) and it is reasonable to conclude that the uptake of ^{42}K across the cochlear partition is energy dependent

According to Ullrich (1974), the net flux ΔJ, can be expressed by the following equation

$\Delta J = J_{\text{solvent drag}} + J_{\text{diffusion}} + J_{\text{active}}$

In the steady state, $\Delta J = 0$

Therefore $-J_{\text{active}} = J_{\text{solvent drag}} + J_{\text{diffusion}}$

The $^{+}$K leakage flux from the endolymph to perilymph can be assumed to be either flux caused by diffusion or a combination of diffusion and solvent drag Presumably $^{+}$Na flux from the perilymph to endolymph is passive These interpretations are quite in agreement with the findings that anoxia or local application of ouabain results in an increase in the concentration of $^{+}$Na and a decrease of $^{+}$K concentration in the endolymph (Mendelsohn & Konishi, 1969, Konishi & Mendelsohn, 1970)

It is difficult to determine the location of the active transport, because of the morphological heterogeneity of the cochlear partition However, it is evident from the results obtained by the simultaneous perfusion of the scala vestibuli (or tympani) with "cold' solution and perfusion of the scala tympani (or vestibuli) with "hot" solution that the lateral wall of the cochlear partition is responsible for active uptake of ^{42}K and extrusion of ^{22}Na and that Reissner's membrane does not play a major role in the active ion transport Jahnke (1975) reported on the fine structure of the freeze-fractured intercellular junction in the cochlea of guinea pigs and found that the intercellular space of the stria vascularis is sealed to the spiral ligament by a very tight junction of basal cells Kuijpers (1969) reported that the highest activity of Na–K activated ATPase was found in the stria vascularis of the guinea pig The oxygen consumption of freshly dissected stria vascularis is high too, according to Chou & Hughes (1964) In conjunction with these findings, our results suggest that the stria vascularis is a site of active transport The marginal cells are the only cells in the stria vascularis that are contiguous with the endolymph and characterized by basal infoldings and large dense mitochondria These are characteristic for cells of other organs involved in ion transport (Pease, 1956) It is likely that the
cells may play an important role in th
transport of $^{+}$K and $^{+}$Na

Compartmental analysis has prove
when applied to problems pertaining
trolytes and water movement in th
(Llaurado, 1973) Recently Mnich (19
compartmental analysis and estima
efflux of ^{24}Na in isolated cochlea from
brane section containing the stria va
into artificial endolymph The compar
model must be consistent with what is
about the physiological configuration
system under consideration The end
is absorbed by certain tissues along th
lear partition, even though the questio
the site of absorption remains unansw
this respect a three compartment syste
nearly represents the physiological co
tion of the cochlear fluids than does t
compartment model used in this study
the compartments are identified with th
media and perilymphatic space as pre
described The third compartment c
identified with the tissues which abs
endolymph This third compartment
considered to be open, since vasculariz
that compartment may result in the tr
of ions out of the regions of interest
open three compartment system is u
modelling the transport of ^{42}K from th
lymph to the endolymph it is found t
uptake of ^{42}K is represented by the sum
exponentials Our results show that the
thesis that ^{42}K uptake in the endolymph
presented by a single exponential cann
tistically be rejected at the $p = 0\ 05$
Therefore the absorption of the endo
does not seem to be a dominant factor in
experiments and the closed two-compa
model can be used to reproduce the k
of the uptake of ^{42}K in the cochlear fluid

The transport rate constant obtained
compartmental analysis is based on t
sumption that the concentration of ^{42}K
stria vascularis is of the same order of
tude as that in the scala tympani Presu

former is slightly higher than the latter and actual transport rate constant for K may y slightly from that calculated As noted in (8), a slight difference in C_{in} caused a large iation in the transport rate constant Thus, ermination of the transport rate constant $^+$Na requires a precise value of the concentration of $^+$Na in the endolymph It is difficult estimate the transport rate constant for $^+$Na ng data available in literature A more accurate determination of $^+$Na concentration th sensitive equipment is needed

The dynamic equilibrium of electrolytes in cochlear fluids is maintained by diffusion, nosis, bulk movement owing to hydrostatic ssure and active transport The results described here demonstrate our first step to icidate the mechanism of electrolyte exchange between perilymph and endolymph ile maintaining a concentration gradient across the endolymph—perilymph barrier ir results illustrate contrary to findings by uch et al (1963) that the stria vascularis is e site of active ^{42}K transport into the endolymph and ^{22}Na extrusion The cochlear partition is permeable to both $^+$K and $^+$Na An understanding of the mechanism controlling omeostasis in the cochlear fluids is essential studying the pathogenesis of various hearing impairments which may occur as a result of e disturbance of the dynamic equilibrium of ectrolytes in the cochlear fluids

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ZUSAMMENFASSUNG

e Scala vestibuli und/oder tympani von betaubten eerschweinchen wurden mit kunstlichem Perilymph elches ^{42}K und ^{22}Na enthielt für Zeitdauern von 5 bis 60 inuten behandelt Die wahrend der Behandlung durch hall hervorgerufenen Reaktionen wurden protokolliert e im Perilymph und Endolymph enthaltenen Aktivitaten urden mit Hilfe eines Germaniumdetektors und eines Mehrkanalanalysators bestimmt Die Behandlung der Scala vestibuli und/oder tympani hatte keine nennenswerten Veranderungen der elektrischen Reaktionen zur Folge Die Eingabe von ^{42}K und ^{22}Na in das Perilymph der Scala tympani verursachte das fast sofortige Erscheinen dieser Radioisotopen im Perilymph der Scala vestibuli Die ^{42}K und ^{22}Na Aktivitaten im Perilymph der Scala tympani verblieben niedrig beim Behandeln der Scala vestibuli Dies stimmt mit unserer Entdeckung uberein daß das Abklingen dieser Radioisotopen in der Scala tympani schneller vor sich ging als in der Scala vestibuli Unsere Resultate zeigen außerdem daß sich das Endolymph mit ^{42}K anreicherte wahrend es ^{22}Na abschied und zwar gegen die Konzentrationsgradienten Der Transport von ^{42}K und ^{22}Na durch die Cochlea Trennwand wurde durch Anoxia oder durch lokale Anwendung von Ouabain verhindert Das Anreichern von ^{42}K im Endolymph vom Perilymph der Scala vestibuli war vergleichbar mit dem Anreichern vom Perilymph der Scala tympani Diese Resultate deuten darauf hin daß $^+$K aktiv vom Perilymph zum Endolymph transportiert und $^+$Na vom Endolymph zum Perilymph abgeschieden wird und daß der aktive Transport von $^+$K in der Stria vascularis stattfindet Die Transportgeschwindigkeits Konstante von $^+$K wurde von unseren experimentellen Werten mit Hilfe eines angenommenen Zweizellensystems berechnet

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GENTAMICIN-INDUCED MITOCHONDRIAL DAMAGE IN INNER EAR SENSORY CELLS OF THE LIZARD CALOTES VERSICOLOR

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stract Earlier morphological and histochemical studies the effects on inner ear sensory cells caused by amino-oside antibiotics have failed to give sufficient informa i of the genesis of these effects The present study , focused on the phases of progressive mitochondrial .nges in sensory cells of the the lizard basilar papilla -uced by consecutive large doses of gentamicin The ochondria react by swelling changes in the configura i and number of the cristae and formation of matrical lusions Myelin figures are a consistent finding in de ierating cells after gentamicin exposure These are ›wn to be derived from changed mitochondria The ,al product is an onion like structure which is built of mitive membranes There is a marked difference in ction to the damage between individual mitochondria the same cell This difference is also evident between ividual sensory cells in the same specimen By study the phases of the mitochondrial breakdown process the sensory cell some additional information on the inges in cell metabolism caused by ototoxic drugs y be extracted

nce the early description of the mitochon-ial ultrastructure by Sjostrand (1953) and lade (1952 1953) evaluation of changes in ced in mitochondria by various agents has en an important task in attempts to clarify : process of cell degradation and death irough the investigations of Friedmann & rd (1961) and Wersall & Hawkins (1962), rk inclusions have been found in inner ear nsory cells of streptomycintreated animals iese bodies were thought to emanate from tochondria In several studies on inner ear mage caused by noxious agents such as otoxic antibiotics sound trauma etc , signs degenerated mitochondria have been pres-t To our knowledge, however no detailed analysis of the gradual development of such changes have been made As important reactions related to the electron transport chain, synthesis of proteins and storage of DNA and RNA take place within elements of the mitochondria, an alteration in structure probably reflects an alteration or impairment in func tion As the mitochondria seem to be susceptible to damaging external stimuli, their structural state, in some aspects, can be taken as a parameter of the general condition of the cell

The aim of the present paper was to investigate the effect of an aminoglycoside anti biotic, gentamicin, upon mitochondrial structure in the sensory cells of a model organ, the basilar papilla from a lizard, *Caoltes versi color*

MATERIAL AND METHODS

The lizard *Calotes versicolor* was chosen as the experi mental animal The animals were kept in a humid en vironment at about 27°C Gentamicin an antibiotic be longing to the aminoglycoside group (Schering GMC 3 M 675) was administered intraperitoneally in doses of 150 mg per kg bodyweight per day A few animals in the first test group received only 100 mg gentamicin per kg bodyweight per day The morphological changes in these latter animals were the same as in the rest of the subjects of the experiment The intraperitoneal administration was chosen for delivering the drug Since the lizard middle ear forms a wide-necked recess from the epipharynx intra tympanal administration is not suitable In order to study the direct and the persistent effects of the drug the test animals were divided into two groups The animals of the first test group were given the drug for 7 to 21 days and

sacrificed one day after the last injection The second test group of animals were injected for 3 or 5 consecutive days These lizards were sacrificed 21 days after the last injection 26 animals were included in the first group and 7 in the second After sacrificing the animals the skull was rapidly bisected and the medial aspect of the temporal bone was removed The membranous labyrinth was

ing to current methods (Rhodin 1954 Wersall 1956 Luft 1961 Reynolds 1963) and mounted on one hole copper grids The sections were examined in a Siemens Elmiskop I or Jeol 100C transmission electron microscope

RESULTS

Normal anatomy

The basilar papilla, the hearing organ of the lizard, is an oblong organ about 425 μm in length It harbours about 225 sensory cells divided into two populations The ventral pop on consists of about 50 cells These cells equipped with short sensory hair bundles they are unidirectionally polarized (Bag Sjoback and Wersall, 1973) A tectorial membrane covers this cell population The dorsal cells have tall sensory hair bundles and are bidirectionally polarized This population has not been shown to have a distinct covering *The sensory cells are cylindrical or flask shaped and have a length of up to about* 25–45 μm (Bagger Sjoback 1976)

Mitochondria are present throughout the cytoplasm and are especially abundant close to the plasma membrane in the area below the cuticular plate and in the infranuclear part of the sensory cell They are also often present close to the synaptic areas No mitochondria are normally seen in the sensory hairs or cuticular plate

Mitochondrial structure The mitochondria show variations in shape and size within the sensory cells Spherical or ovoid and elongated forms are common Although most mitochondria have a diameter of about 0 5–1 μm some can be up to 4 μm in length (Fig 1)

Most mitochondria have the orthodox configuration with the outer compartmen defined and the matrix more electron than the cytoplasm The configuration cristae is somewhat variable even with same cell Some cristae extend over the diameter of the mitochondrion (Fig 2) , angulation of the cristae may appear treated sensory cells In the elongated chondria the cristae are often arranged tudinally (Fig 3) Matrix granules may singly and rarely, there are two or mo mitochondrial cross section

elongated mitochondria dominate parts of a se s in the left lower part of the micrograph The senso

(N)

Fig 3 Normal elongated mitochondrion in an un animal Note how the cristae traverse the whole of the mitochondrion An afferent nerve ending (N synaptic area is also present

Fig 4 Early degenerative phase with swollen m

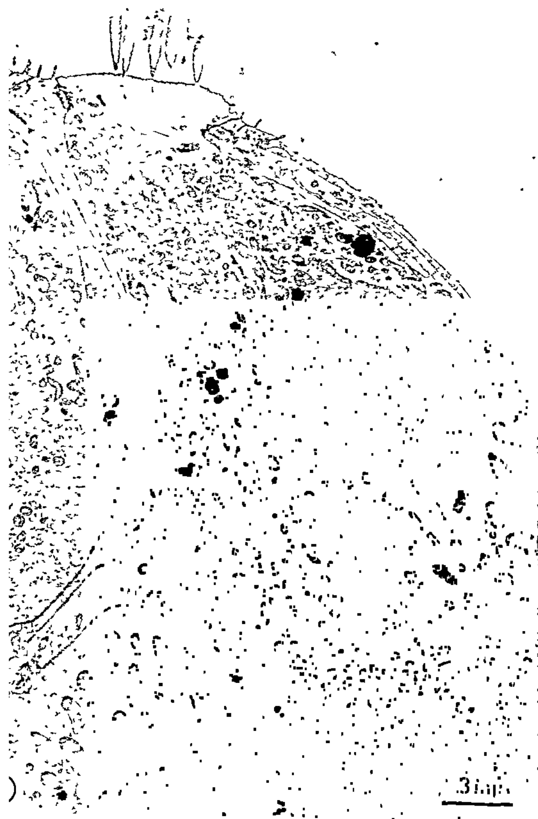
characterized by irregular karyoplasm as a

ment 8 days

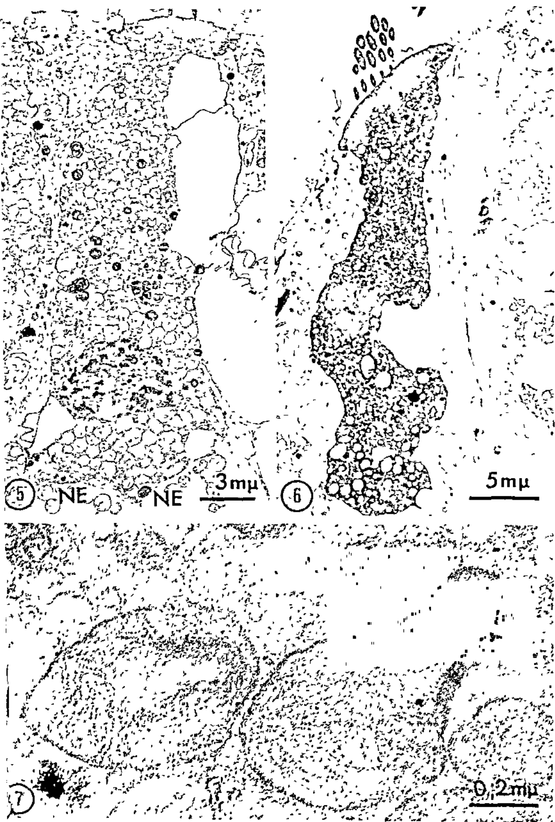
Fig 6 Darkly staining sensory cell with irregular ou The nucleus is degenerated and the cytoplasm minated by vacuoles and mitochondria in state of a tion Compare the features with those of the senso *to the right Gentamicin treatment 8 days*

Fig 7 Mitochondria from the dark staining senso in micrograph No 6 The left and central parts

These lamellae have the regularity of a ost myelin sheath thus this phase seems to be the tion from morphologically normal mitochondria to a or a myelin figure Gentamicin treatment 8 days


3 μm


2
N
0.5m
NE
3
0,5mμ
N
2mμ


5
NE
NE
3mμ
6
5mμ
7
0,2mμ

Changes in mitochondria after gentamicin intoxication

Direct effects Seven days of treatment with gentamicin in some sensory cells may result in vacuolization, however the individual variation is great Many cells appear normal while others show typical signs of degeneration The mitochondria usually have a normal distribution A *swelling* of the mitochondria in the changed cells results in a rounded or ovoid instead of an elongated appearance Some *electron-dense inclusions* the size of mitochondria may also be seen in some sensory cells (Fig 4) A marked increase of the *matrix granules* is evident (Figs 9, 10, 15) The mitochondrial *cristae* are often crescent-shaped and arranged *in concentric rows (Fig 14) These general* changes became more pronounced during the consecutive days of treatment In the more severely damaged basilar papillae, the variation between changes in individual sensory cells is striking Large intercellular vacuoles compress and distort the outlines of the cells (Fig 5) Most mitochondria are , and rounded and only rarely are elon- *forms seen Some sensory cells are* almost completely filled with mitochondria in different stages of degeneration (Fig 5) Electron dense *myelin figures* may be present singly or in aggregates but do not dominate the field Most mitochondria have the orthodox configuration while some are more or less empty with only few cristae remaining (Fig 8) Other cells have a pronounced electron density (Fig 6) and contain mitochondria, in what appears to be a transformation phase In other sensory cells most mitochondria appear swollen and show a loss of inner structures (Fig 8) Few, if any, cristae remain and the matrix appears empty

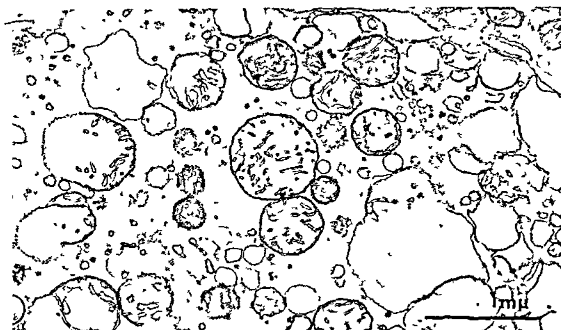
By studying mitochondria from different parts of the same sensory cell and different sensory cells in the same basilar papilla, a certain pattern of mitochondrial degeneration can be observed Apart from a varying degree of swelling, the mitochondrial cristae in some areas of the mitochondria loose their s contours A dense substance accumulate tween the membranes of the cristae an tween the cristae until a blurred area is fo in the mitochondrion Later, parallel la with equal intervals appear in this dark stance, so-called myelin lamellae Thus area of the mitochondrion can be compl altered, whereas another area can still co normal-appearing cristae The changes ceed until finally the whole mitochondri

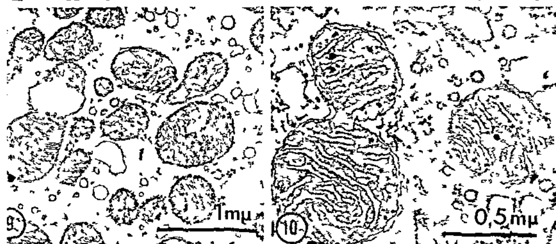
Fig 8 Rounded swollen mitochondria with marke of internal structures Gentamicin treatment 8 days
Fig 9 Disruptured mitochondrion with hem a

like structures are present

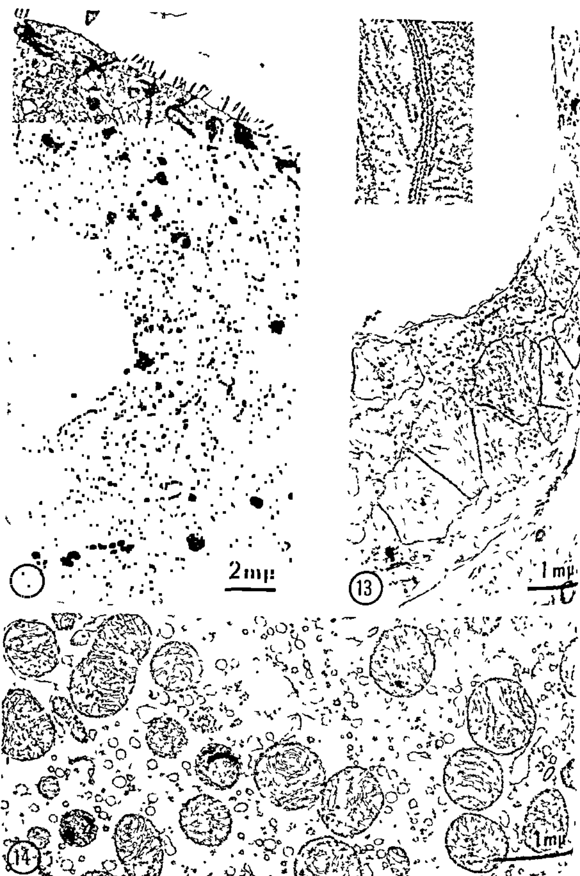
membranes with signs of fine interconnecting fila Gentamicin treatment 14 dyas

seems to separate it into two compartments Ge treatment 14 days
Fig 15 Waist lined mitochondrion with 7 distinct ma inclusions in the section Note the angulations o cristae and the beginning lamellar arrangement i organelle in the right lower part Gentamicin treatm days The inset shows a mitochondrion with a lar clusion that seems to dislocate the cristae Genta treatment 11 days
Fig 16 Large complex mitochondria with internal a gates of rounded or spiral shaped cristae Genta treatment 11 days
Fig 17 Advanced cellular degeneration with rou swollen mitochondria and several myelin figures gregates of two or more disintegrating organelles ca seen contained within a membranous bag" T bags are thought to have an autophagocytotic act Gentamicin treatment 13 days


1mµ

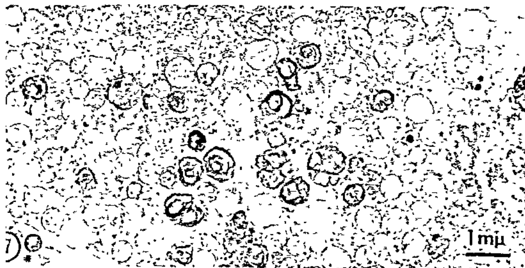

9
1mµ
10
0,5mµ


11


2 mμ
13
1 mμ
14
1 mμ


1mμ


1mμ


1mμ

changed into a dark body richly provided with myelin lamellae (Fig 7) The thickness of the lamellae in the osmium fixation is about 70 Å (Fig 7, inset) In some sensory cells "onion-like" structures with or without a central core of an amorphous material and the same size as the mitochondria make the appearance (Fig 11) These structures become more abundant as the cell changes progress After further treatment the mitochondria may show a *partial vacuolization* where some parts appear normal and others swollen *Matrix granules* are abundant (Figs 10, 15, 16), often seven or more granules are seen in one mitochondrial cross section (Fig 15) Most granules are round with a diameter of about 200–300 Å, but some reach a diameter of 0 5 μm and displace the cristae (Fig 15, inset) The latter type of inclusion however is rarely seen in the sensory cells Some mitochondria are indented and may be nearly bisected (Fig 10) Often a bridging crista is seen in the narrow waist (Fig 14) *Angulated cristae* are seen in the treated as well as in the untreated ones How- the degree of angulation in the treated is greater and may exceed 90° These lations sometimes render a zig-zag appearance to the cristae (Figs 14, 15) Angulation of cristae is common in mitochondria of long term treated animals (Figs 10, 11, 14, 15, 16) Circular or crescent shaped cristae are rare in untreated animals but common in treated specimens, particularly in animals treated 10 days or more (Figs 11, 14, 16) Often several spirals or aggregates of cristae are present in the same mitochondrion (Fig 16) In some mitochondria the cristae may even be branched

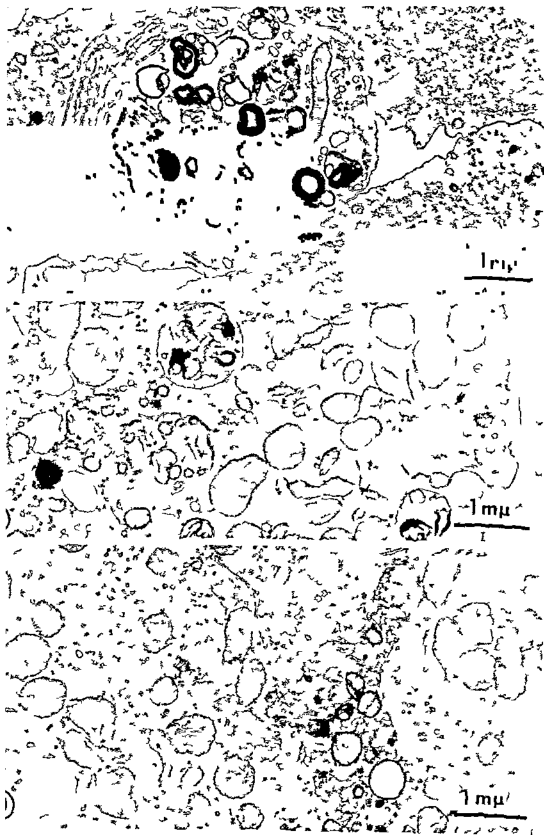
In animals treated for about 2 weeks, signs of more severe damage are present throughout the basilar papilla Large intercellular vacuolization, protrusions from the cellular surface into the endolymphatic space and fusion of the hairs are common The sensory cells are distorted and the cytoplasm contains a rich supply of small vesicles (Fig 12) Most mitochondria are swollen and in different stages of degeneration The matrix has a lower deg electron density and elongated forms ar Myelin figures and "onion like structur common Concentric membranes are throughout the cytoplasm (Fig 17) an contain cellular material Aggregates of m figures or cellular debris are often enclo a membrane (Figs 17, 18) Such structur interpreted as autophagocytotic and the in size and shape Often remnants of elles are seen in them (Figs 17, 18) In specimens, mitochondria have a tender establish a direct contact with one anoth the round mitochondria only a part of t riphery is involved In other cases the chondria appear angulated and more o square These mitochondria are in in contact with each other (Fig 13) The membranes of two adjacent mitochond not fuse but leave a gap This gap has stant width of about 140 Å In the osmium tion the gap seems to be traversed by fi ments (Fig 13, inset)

After treatment for over 2 weeks the b papilla is severely damaged The sensor may be largely absent and with progressi generation, the surface of the basilar p becomes dominated by the supporting The sensory cells can be detected belo organ surface and are dominated by phagocytotic bags containing aggregat debris, membrane whorls or myelin fi and amorphous structures Even in th

Fig 18 Severely damaged remains of a senso Membranous "bags containing cellular debris and figures dominate the field However fairly nor

the second test group showing persistent effec mitochondria with angulated or semicircular cris evident as well as aggregates of cellular debris and figures contained in autophagocytotic bags" Gen treatment 3 days with 21 days latency

Fig 20 Short term animal of the second test gro sensory cells in much the same state as in fig 19 S irregular mitochondria and a large aggregate of debris is present Gentamicin treatment 5 days w days latency


1 mμ
1 mμ
1 mμ

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Fig 18 Severely damaged remains of a senso Membranous bags containing cellular debris and figures dominate the field However fairly nor pearing mitochondria and endoplasmic reticulum present Gentamicin treatment 15 days
Fig 19 Degenerated sensory cell in short term a the second test group showing persistent effects S mitochondria with angulated or semicircular cris evident as well as aggregates of cellular debris and figures contained in autophagocytotic bags Gen treatment 3 days with 21 days latency
Fig 20 Short term animal of the second test gro sensory cells in much the same state as in fig 19 S irregular mitochondria and a large aggregate of debris is present Gentamicin treatment 5 days days latency

As the main task of the sensory cell nsduce mechanical stimulation at the hairs to nerve impulses in the afferent ystem it seems reasonable that the ytoplasm near the roots of the sensory d the cytoplasm close to the synaptic ave certain metabolic needs This can said about the region adjacent to the membrane

ondrial swelling and early cristal

;

ondrial swelling is an early sign of de- ion and may represent an unspecific in the ion balance across the mitochon- embranes Persistent swelling may be a change in metabolic activity (Dow, Several toxic agents have been reported ce swelling of the mitochondria as one first signs of damage Inhibitors of nu- cid replication and protein synthesis s chloramphenicol and ethidium bro- roduce swelling and loss of cristae in ondria of treated cells (Lenk & Pen- 971, Howell et al , 1971, Hwang et al , Mitochondrial swelling and loss of cris- inner ear sensory cells was noted by & Igarashi (1969) after treatment with cin sulphate They concluded that the iad penetrated into the cells from the ial surface and that the mitochondria he initial sites of destruction The swell- the mitochondria in the present material ed upon as a rather nonspecific change early stages it might be reversible In very swollen mitochondria, a loss of the may occur in the early phase of cellular after gentamicin treatment This type of hondrial change probably involves an icity of oxidative phosphorylation and ially of respiration (Trump & Ginn

n figures

enesis of myelin figures is under debate dless of their origin they are charac c of dying and dead cells (Trump & Ginn, 1969) Whorls or myelin figures in inner ear sensory cells treated with aminoglycoside antibiotics were first described by Friedmann & Bird (1961) and Duvall & Wersäll (1964)

Lundquist & Wersäll found such whorls in inner ear sensory cells after treatment with kanamycin (1966) and gentamicin (1967) The whorls or myelin figures in the sensory cells of gentamicin treated animals are considered to emanate from degenerated mitochondria, as evidenced from the finding that one pole of the mitochondrion can appear morphologically normal while the opposite pole consists of a membranous whorl Similar progressive tran- sitions have been detected in mitochondria of the aging blow fly (Sachtor & Shimada, 1972) It was shown that the cytochrome oxidase activity was significantly decreased in the part of the mitochondria dominated by the mem- branous whorls The final stage of breakdown seems to be a darkly staining multilamellar body or an "onion like" structure

The earlier material presented by Duvall & Wersall (1964) and Lundquist & Wersall (1966, 1967), did not show sequences of developing changes in the mitochondria leading to myelin figure formation In the present material we have been able to follow different steps in the degeneration of mitochondrial cristae by com- paring changes in neightbouring mitochondria in the same cell as well as mitochondria in different cells in the same sensory epithelium During this degeneration, the sharp limits of the membranes of the crista disappear A dark substance is formed at the site of the degen- erated cristae, and finally the membranes are soon to fuse forming parallel dark lines with an interval of about 70–80 Å It seems likely that these myelin figures appear through de generation of the phospholipid and protein complex of the cristae membranes with forma tion of new unit membranes arranged in a way similar to that of a myelin sheath The multi- lamellar "onion like" structures have the ap- pearance of a phospholipid vesicle as de- scribed by Ansell et al (1973) These might thus represent the final stage in the breakdown

A ta Or lar

process of the mitochondria An interesting observation is that these changes appear very irregularly in a cell Thus some of the mito chondria in a cell seem to be fairly intact, whereas others are completely changed to myelin figures or onion like' structures This may explain why the cells seem to survive a long time even with severe intracellular changes

Matrix granules

Lundquist & Wersall (1967) called attention to the small matrix granules of the mitochondria *in the sensory cells of the guinea pig inner ear* after treatment with gentamicin The nature of these inclusions is still not clear Weiss (1964) and Peachey (1964) proposed that the inclu sions could be sites for the binding of cations Barnard & Lindberg (1969) and Barnard & Afzelius (1972) considered them to contain mostly lipids The very large inclusions found *in some mitochondria are probably not com* posed of cations but rather some kind of lipid erial Presumably the matrix granules in material is a sign of a lipoprotein unmask

Changes in shape and size of mitochondria

Herniations of mitochondria commonly occur in the treated cells It is unlikely that they ap pear only as a consequence of the swelling process but rather that they represent an in creased susceptibility of the outer mitochon drial membrane to the fixation procedure In dented mitochondria commonly seen in many cells may be a sign of formation of new mito chondria (Larsen 1970) Further support for this idea can be derived from the heavily mito chondria laden sensory cells with moderate damage It appears that the number of mito-chondria may be increased in an attempt by the cell to cope with the decreased enzymatic capacity of the damaged mitochondria An gulations of the mitochondrial cristae have been reported in normal cells by Revel et al (1963) and Slautterback (1965) Luft et al (1962) described angulated and spiral cristae

in muscle mitochondria from a pat ing from hypermetabolism Simi were seen in this study and also in c with chloramphenicol ethidium (Lenk & Penman 1971 Kislev et Tightly aggregated mitochondria as material can be correlated to mo damage The condition has been s retinal cells by Pease (1962) and cells by Pearse & Welsch (1968) A between the mitochondria has bee by Pease (1962) and believed to be fine joining filaments

CONCLUSION

It is important to emphasize that th action of the aminoglycoside antibi be different in microorganisms and ryotic cells as in the inner ear of ve In microorganisms these antibiotic *impair certain ribosomal function* 1971) Whether a ribosomal system i cells can be affected by aminoglyc biotics is not clear Studies on the cellular RNA in intoxicated cells is u It has been shown that aminoglyc biotics are regarded to be relatively of penetrating the cell membrane (I Sullivan, 1963) and accumulation of in membranes has been demonstrate re (1956) Hence the drugs ought to primarily with the surface structur cell i e the plasma membrane Flock (1964) demonstrated a revers

considered to depend on a blocking membrane function Wersall et al (1 sidered the membrane fusion of sens to depend on an incorporation of g in the plasma membrane thus surface area of the membrane (1976) studied the interaction of on phosphoinositide metabolism in ear of the guinea pig He suggested *mycin is incorporated into the mem*

its properties The area of the plasma
·ane would thereby be altered This
support the thought that fusion of sen
airs into giant formations may be ex-
l by changes in membrane area The
n and phosphorous incorporation in the
·ane is connected with the phospholipid
olism and molecular array, and this
g of calcium seems to be altered under
luence of the drugs The aminoglycoside
nicin is regarded by Schacht to share the
; on membranes with neomycin, and to
re reactive in the cochlear tissue than in
estibular tissue We however, regard
nicin to be strongly toxic to the vesti
system as well The theory of a direct
of the antibiotic on the plasma mem-
with a secondary leakage of the drug
ie cell supports the findings of Bagger-
:k & Wersall (1976) in their study on
nicin toxicity in the lizard basilar papilla
lorsal sensory cell population with long
ry hairs has a larger surface area than
entral one This dorsal cell population
resented a more severe damage pattern
:he ventral one This can be explained by
e rapid penetration of the drug into these
due to their larger surface area The steps
generation of hair cell mitochondria in the
nt study indicate that gentamicin may
iave a direct action on the mitochondria
: hair cells
ias been established by several authors
lrugs do not introduce different structures
nctions in the cell but alter the existing
vays of metabolism Thus many toxic
:ances can be thought to act through a
non morphologic pathway of degenera
(Forbus 1943, Trump & Ginn, 1969)
chondrial changes have long since been
vn to be a prominent feature of the de
rative process in the cells As mitochon
are known to contain an organelle specific
ι and RNA (Nass et al 1965) they can be
ımed to enjoy a certain degree of auto-
y within the cell The inner mitochondrial
ıbrane contains the respiratory enzymes
as well as other important enzyme systems, thus mitochondrial degeneration in the sensory cells after administration of gentamicin will severely change the functional state of the cell In order to clarify the specific site of action of the antibiotic, histochemical and biochemical studies should be made Further studies are also required to clarify whether or not other metabolic systems in the cell are affected by the antibiotic Such studies are under way

ACKNOWLEDGEMENT

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ZUSAMMENFASSUNG

Fruhere morphologische und histochemische Studien uber die Wirkung von Aminoglykosid Antibiotika auf die

Anschwellung Veranderungen in der Konfiguration und Anzahl von Cristae als Bildung von Inklusionen in Matrix Myelinfiguren sind regelmassig sich wiederholende Beobachtungen in degenerierenden Zellen nach Gentamicinexposition Deren Hauptteil stammt aus veranderten Mitochondrien Das Endprodukt ist eine zwiebelahnliche

Studien der verschiedenen Phasen im Abbau der Mitochondrien in den Sinneszellen konnte eine weitere Information uber die Veranderungen im Zellenmetabolismus verursacht durch ototoxische Drogen erzielt werden

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ORGAN CULTURE OF THE 16TH GESTATION DAY MOUSE LABYRINTH

A Model Suggestion for Pre- and Post partum Development

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(Received October 25 1977)

Abstract The 16th day mouse embryo inner ear was explanted and cultured *in vitro* for 6 days which corresponds to 1 day post partum development *in vivo* A normal development occurred both when the inner ear anlage was divided into a vestibular and a cochlear portion and when it was cultured as a whole The present model system may provide a helpful tool in the study of both normal development and inner ear pathology

Successful organ culture of a mammalian inner ear was first reported in 1971 by Van De Water & Ruben Several reports have thereafter been ... lished applying this organ culture system ... analysis of embryological mechanisms ... in normal development (Van De Water ... Ruben 1973, Li et al , 1976, Van De Water 1976, 1977) and abnormal development (Van De Water & Ruben 1971) These experiments utilized eleventh, twelfth and thirteenth gestation day mouse embryo inner ear anlage and concentrated primarily upon early embryological development

This study was designed to provide a model system for the late stages of embryological development and the post natal period of development Preliminary organ culture results are presented in developing a late pre and post partum model system

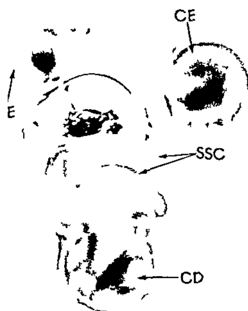
MATERIALS AND METHODS

CBA/CBA mice were staged for gestational ages by the vaginal plug technique with the day of plug being designated as gestation day one Gravid females were sacrificed on the 16th day by cervical dislocation and th... removed and placed in Dulbecco s phos... buffered saline (PBS) The embryos w... moved from the uteri and rapidly deca... with a fresh razor blade knife The e... heads were then dissected along the... tudinal midline and the brain tissue re... The exposed labyrinthine capsule w... moved from the temporal bullae with... ened watchmakers forceps The expla... cleaned of surrounding tissues with the... makers forceps and no 25 gauge hypo... needles that were used as microscalpel... resultant 16th day embryo ear was eit... planted whole or cut in half at the level... ductus reunions between the saccule a... cochlear duct, leaving two explants—a... bular explant and a cochlear explant T... plants were placed in Falcon plastic... culture dishes that were coated with... agar in media solution as a substrate an... filled with 0 25 ml of organ culture m... Neumann and Tytell s serumless m... supplemented with 1% l glutamine ... pyruvate and 10% fetal calf serum Th... perature of incubation was 35°C and t... trient medium was renewed every thi... of culture

Following 6 days in culture the ex... were fixed with a 2% solution of osm...

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L ght microscopy (LM) Otocyst explanted on the stat on day and cultured for 6 days in vitro *Top* ocyst d v ded at explantation nto a cochlear end nd a vest bular end (*VE*) *Bottom* Whole otocyst chlear duct *SCC* sem circular canals ×20

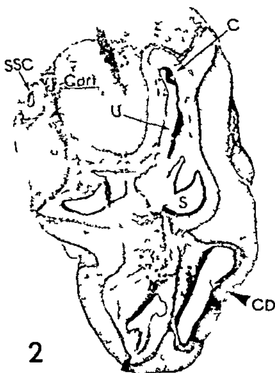


Fig 2 LM Otocyst explanted on 16th gestation day and cultured for 6 days in v tro Paraffin section through the whole explant *C* crista ampullaris *U* utricle *S* saccule *SCC* semicircular canals *CD* cochlear duct *Cart* cartilage ×40

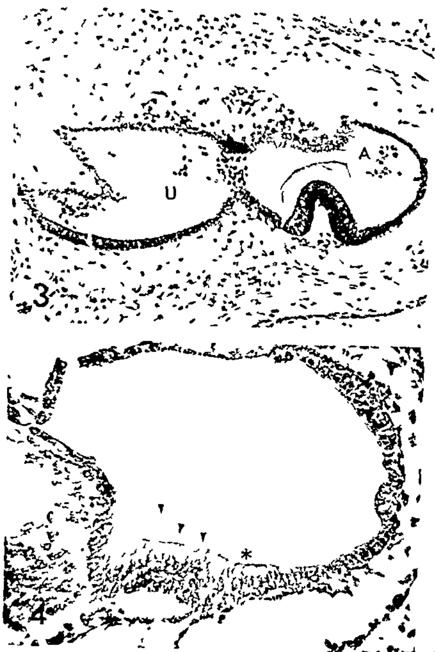
lium phosphate buffer (pH 7 2–7 4) for 1 After a rinse in buffer and dehydration pecimens were embedded in paraffin and or serial sections of the whole inner ear ing haematoxylin eosin)

RESULTS

n culture explants were examined daily a Nikon dissecting microscope The 16th explants at the time of explantation already undergone their most dramatic phogenesis The only grossly observable nges were a lengthening and increased ng of the semicircular canals

microphotograph of living whole ear and ibular and cochlear explants after 6 days n vitro development is seen in Fig 1 A histological cross section of one of the whole ear explants is illustrated in Fig 2 These ex plants showed differentiation of all of the vestibular sensory structures and base to apex pattern of differentiation of the cochlear duct The vestibular sensory epithelium differen tiated in all of the explants containing vesti bular structures and sensory hair bundles and cupula formed in the explants (Fig 3) The cochlear duct sensory areas differentiated in the explants that contained these structures The basal turn of a cochlear duct with Corti s organ of an explant after 6 days in vitro is illustrated in Fig 4

This preliminary group of explants was composed of 15 specimens and a similar level of differentiation was observed in all explants as is depicted in Fig 1 to 4



Figs 3 4 LM Paraffin se
Otocyst explanted on 16th
day and cultured for 6 day
×90 *Fig 3 A* crista amp
The cupula is formed and
hair bundles are recognizal
utricle *Fig 4* Basal coil of
mouse cochlea The tecton
membrane is indicated by a
The location of cochlear ha
is marked by ×

DISCUSSION

Ruben (1967) reported that most cells that will form sensory cells in the mouse inner ear have undergone their terminal mitotic divisions by the 16th day of gestation The work of Sher (1971) has shown that the 16th gestation day mouse embryonic inner ear is beginning to differentiate vestibular sensory structures but has not yet begun to differentiate cochlear duct sensory structures The developing peri lymphatic spaces of the 16th day embr are encased in the cartilaginous ca Therefore organ culture of the 16th embryonic tissue interactions involved ferentiation may have occurred The bility of more prolonged organ culture be realized due to the presence of the d oping perilymphatic spaces which may vide access of the nutrient medium to developing sensory structures of the lymphatic lumen

presented in the present study it is pos- short term culture the 16th day embry- ouse inner ear and have a normal devel- t No adverse effects were observed as t of the dissecting of the explants into llar and cochlear portions Studies are nder way to establish for how long a of time the vestibular and the cochlear ts can differentiate and then be main in the organ culture environment If attempts at long term culture are suc- l this model system might be employed dy many experimental pathological as s hereditary diseases that previously only be studied in vivo The model may e employed in testing the effect of oto- drugs on an isolated developing laby

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nenohr des sechzehntagigen Mausembryos wurde oben und 6 Tage in vitro kultiviert welches der klung eines Tages post partum in vivo ent Eine normale Entwicklung folgte sowohl wenn enohranlage in eine vestibulare und eine cochleare Partie geteilt wurde als auch wenn sie als ganzes kulti viert wurde Das gegenwartige Modellsystem erbietet ein gutes Hilfsmittel für das Studium sowohl der normalen Entwicklung wie der Pathologie des Innenohres

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DIFFERENTIAL EFFECTS OF PRIMARY FIXATION WITH GLUTARALDEH AND OSMIUM UPON THE MEMBRANOUS SYSTEMS OF THE STRIAL A\ EXTERNAL SULCUS CELLS

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Abstract Ultrastructural preservation of the lateral cochlear wall differs between primary osmium fixation and primary glutaraldehyde fixation Of significance in this study was the fact that whereas glutaraldehyde fixation preserves the parallel plasma membranes of interdigitations of the strial and external sulcus cells fixation with osmium occasionally produces a breakdown of selected areas of membranes into apparent vesicles The occurrence of these membrane artifacts was more common within the external sulcus cells Reasons for the differential effects of the fixatives may include their differing modes of action Care should be taken when evaluating unusual ultrastructure since the type of fixative employed may be causal

roscopy is a frequently used and tool for the study of the auditory apparatus Ultrastructural detail is of critical consequence in the interpretation of both normal and pathologic cellular processes It is not surprising, therefore, that artifacts introduced by tissue preparatory techniques are highly undesirable and can seriously interfere with the validity of experimental results

For example, most inner ear structures are quite sensitive to the amount of time elapsed from the animal's sacrifice to the application of fixative Wersall et al (1965) have shown that if the interval is greater than 15 minutes, statistically significant ultrastructural changes occur within the organ of Corti Such knowledge is invaluable for the proper ultrastructural interpretation of electronmicrographs

Other less apparent factors may influence fine structure In particular, certain fixatives are able to selectively alter cellular details Glutaraldehyde and osmium tetroxide are perhaps the most widely used fixat f tronmicroscopy, either with glutarald the primary fixative and osmium as fixative, or with osmium alone Quite ent ultrastructural anatomy may be with these two fixation procedures i used upon specific tissue types

The stria vascularis is an extremel tant extrasensory organelle within tl lear duct, whose function is the pr and maintenance of both the positive d c potential and the endc unique make up (Smith et al , 1958, 1 Spyropolous, 1959, Bosher & Warre 1971, Fernandez & Hinojosa, 1974) ous researchers have concerned the with the ultrastructural evaluation of in normal (Smith, 1957, Hinojosa Echandia, 1966, Rodriguez Echandia gos, 1965, Spoendlin 1967, Sugar et a and pathologic states (Takahashi, 1971 et al , 1974, Brummet et al , 1977)

In an evaluation of the effects of glu hyde and osmium fixation upon the str laris, Merck et al (1974) concluded variably glutaraldehyde as the primary causes various ultrastructural derang including vacuolation of the mitochond trices, cell shrinkage, and enlargemen extracellular spaces Maunsbach (19 reported alterations of mitochondria structure following glutaraldehyde fix the kidney It was further concluded by et al that the use of osmium tetroxid



Membrane artifacts (*arrows*) apparently budding rallel plasma membranes within the stria vascula ris Note well fixed membranes adjoining artifacts Osmium tetroxide slightly reduced from 43 200

ry fixative produced no artifacts what r This finding however is perplexing the structure of the stria s cellular com its is considered Like some other spe ed secretory epithelia found within the the stria s marginal intermediate and cells exhibit numerous plasma mem interdigitations Such interdigitations lso found within other epithelia of the ear duct the ciliary epithelium of the and the proximal convoluted tubules of idney In this context it should be noted Tormey (1964) found that fixation of the y epithelium with osmium tetroxide as rimary fixative frequently resulted in the fragmentation of the plasma membrane infoldings to form rows of small clear vesicles Such a finding was absent in glutaraldehyde fixed ciliary epithelium Maunsbach (1966) evaluated osmium and glutaraldehyde fixation in the kidney and also found that adjacent parallel plasma membranes of the proximal tubule cells sometimes formed rows of small tubular or vesicular bodies in cross section Again the occurrence of these structures was dependent upon a primary fixation with osmium tetroxide Other parallel membrane systems in different tissues also respond in like manner to osmium fixation These include the smooth ER of testicular interstitial cells (Christensen



Fig 2 Membrane artifacts (*arrows*) close to endolymphatic surface (*E*) in stria vascularis Osmium tetroxide, ×50300

& Fawcett, 1961), of gastric parietal cells (Ito, 1961) and in the T system in muscle (Franzini-Armstrong & Porter, 1964)

Since the interdigitations of the strial cells are composed of parallel membranes, it is di-

stressing that Merck et al. reported ferences between glutaraldehyde and os fixed strial membranous interdigitations distress is further evoked when one co that various researchers have drawn ar



3 Membrane artifacts (*arrows*) with n external sulcus
I Osmium tetroxide slightly reduced from 42 700

he ex stence of small clear vesicles occa
ally found among the interd gitations of
cells of the lateral cochlear wall For ex
le Smith (1957) in her ultrastructural de
ption of the stria vascularis and spiral pro
ence noted the occurrence of rows of
ll beaded vesicles apparently originating
n the infolded cell membranes of the stria
ilar vesicles were found among the interdi
tions of the external sulcus cells Inter
ngly the fixative employed was Dalton s

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lted that the basal compartments of mar ginal cells contain a variable number of vesicular structures Whether or not these vesicles were contiguous with parallel plasma membrane infold ngs is not known since no electronmicrographs of the vesicles were shown Duvall (1969) found similar small clear vesicles in contiguity with parallel membranes of the external sulcus cells which he interpreted as micropinocytotic vesicles Again the fixative used was osmium tetroxide To this author's knowledge vesicles of this nature within the inner ear have not been reported to occur with the use of fixatives other than osmium tetroxide The purpose of this study then is to evaluate the effects of osmium and glutaralde

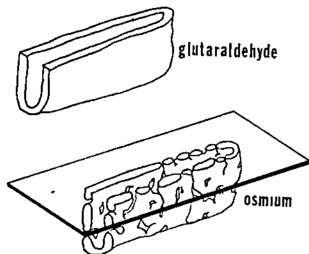


Fig 4 Schematic depiction of artifact formation and visualization with electronmicroscopy (redrawn from Tormey, 1964)

hyde fixation upon the parallel membranous systems of the stria vascularis and external sulcus cells, so that sound ultrastructural interpretation may be made

PROCEDURE

ng adult albino mice were used Prior to apitation, the animals were anesthetized with 25 mg/kg Nembutal Following decapitation, the cochleae were excised, fractured open and immersed either in 1% osmium tetroxide in 0 1 M cacodylate buffer or 2% glutaraldehyde in 0 1 M cacodylate buffer, pH 7 4, at 4°C All right ears were fixed in osmium, and left ears in glutaraldehyde The order of fixation was alternated The average interval between decapitation and fixation was 3 min for the first ear fixed and 6 min for the other After 2 hours of osmication the cochleae were washed briefly in cacodylate buffer, dehydrated in a graded series of acetone, and embedded in Epon at 60°C Prior to embedding, the lateral cochlear wall was dissected free of the bony capsule Glutaraldehyde fixation was 2 hours in length, and was followed by post-osmication for an additional 2 hours, after which the same dehydrating and embedding procedure was performed Ultrathin sections were cut with an LKB ultratome using a diamond knife

The grids were stained with satur acetate and lead citrate, and wer with a Zeiss 9S-2 electron microsc

RESULTS

The overall ultrastructural appear primary osmium fixed specimens w to those fixed first in glutaraldehyde prominent artifact of glutaraldehy was mitochondrial vacuolation an Other differences between osmium raldehyde were apparent As exp striae of those animals which we fixed with glutaraldehyde and osmiu exhibit any breakdown of parall membranes into rows of small clea For the most part, membranes ran though rugosities were noted at tim rugosities never approached discret vesicles On the other hand, tissue osmium alone occasionally had rows clear vesicles apparently budding off interdigitation's plasma membranes These vesicle shaped structures were only in isolated areas of plasma mer most interdigitations kept their parall branous structure after primary fixat osmium The isolated areas of artifa not restricted to the deeper portion stria, however, since these vesicle were observed close to the endolymph face (Fig 2)

Whereas the appearance of artifactu cles derived from the plasma membran not noted in all strial sections examir examined sections of the osmicated e sulcus cells contained these vesicles as ed with their interdigitations (Fig 3) As stria, there were well fixed parallel membranes adjoining these vesicles Gl dehyde-fixed external sulcus cells show such membrane breakdown

DISCUSSION

The occurrence of small clear vesicular tures apparently budding from parallel p-

nes of the strial and external sulcus
ems to be the result of primary osmium
since primary glutaraldehyde fixation
d by osmium produced no such struc
'hese observations are consistent with
lts of research on other parallel plasma
ine systems (Tormey, 1964, Mauns
966) In some undetermined manner,
ı causes a breakdown of selected sec
' parallel membranes It should be real-
at whereas electronmicrographs depict
down into rows of vesicles, the prob
ree dimensional nature of the artifact
ıt be vesicular, as was pointed out by
y (1964) (Fig 4) Possibly during primary
tion, at certain points, opposing mem
coalesce to form a sheet of tubules, but
when viewed in cross section, form
of vesicles

nsbach (1966) implied that penetration
: the fixative was responsible for the
ane breakdown, since he found paral-

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m, then penetration rates are not very
nt, having K values of 0 34 and 0 2, re
vely (Hopwood, 1967*a*) That the rate of
ation is the unlikely explanation is evi
d by the appearance of these artifacts
the stria vascularis, a tissue of about 30
width. Indeed, vesicles were observed
to the endolymphatic surface (Fig 2) It
d be recalled that the cochleae in this
were fractured open and immersed in
/e so that the fixative made direct con
vith the striae and did not have to pene
either the basilar or Reissner's mem
: as is the case with the intralabyrinthine
sion technique It is difficult to account
ıe artifacts on the basis of penetration
of the fixatives used

:haps the explanation lies in the differing
ivities of the two fixatives with membra
structural components Whereas glutaraldehyde is noted for its efficient cross linking of proteins, osmium is considered to react mainly with unsaturated fatty acids (Hayat, 1970) The inability of osmium to retain normal structure in the artifact zones points to the possibility that membrane structure (lipidic)—and probably function too—was different at these points The lack of these artifacts with primary glutaraldehyde fixation must be accounted for by its superior protein crosslinking activity It is plausible, then, that primary osmium fixation in some tissue types unmasks areas of specific membrane activity

It is interesting to note the more frequent occurrence of the artifactual parallel plasma membrane breakdown within the external sulcus cells as compared with the striae If indeed the primary osmium fixation is unmasking certain functional sections of membrane, then it would appear that the external sulcus cells' interdigitations have a different amount of activity than those of the striae

It is now clear that the vesicular structures observed by Smith (1957) in the strial and external sulcus cells, and the "micropinocytotic vesicles" seen by Duvall (1969) in the external sulcus cells, were artifacts of primary osmium fixation Spoendlin (1967) reported the frequent occurrence of vesicles within the interdigitations of the prominence stroma cells and outer sulcus cells but not within the stria The type of fixative used for the structures was not specified but presumably osmium was used for the fixation of the first two cell types, and glutaraldehyde was used for the stria The deduction that glutaraldehyde was the stria s fixative can be made because Spoendlin reported the appearance of small clear vesicles *within* the marginal cell processes These vesicles, about 200 Å in diameter, have been found by this author (in preparation) to be microtubules in cross section, and they are only preserved with primary glutaraldehyde fixation

Brummett et al (1977) studied the effects of ethacrynic acid upon strial ultrastructure, and reported that for some of their experimental

animals, small clear vesicles were numerous at *the basal processes of the marginal cells* Since no micrographs of the vesicles were published, it is difficult for the reader to know whether or not these were the result of the fixative used, i e , osmium A lack of vesicles in osmicated controls may not be significant, since not all samples may exhibit vesicles

The differential effects of primary glutaraldehyde and primary osmium fixation on fine structure is important for the proper interpretation of electronmicrographs This is so because effects present with one fixative but not with the other may indicate artifact formation This is especially important for the tissues of the lateral cochlear wall, since generally poor fixation is obtained with glutaraldehyde (Merck et al , 1974) and therefore most ultrastructural work is performed with osmium as the primary fixative Control fixation with glutaraldehyde is rarely, if ever, done Perhaps the undesirable effects of both fixatives alone may be ameliorated by the use of a combined ...ium and glutaraldehyde fixative This pro... has been used with some success ...rump & Bulger, 1966) Until a fixation tech...ique of superior quality is found for use on the lateral cochlear wall, care should be taken in the interpretation of unusual structures present in electronmicrographs

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Die Ultrastruktur der lateralen Cochleawand wird durch primare Osmiumfixation oder primare Glutaraldehydfixation unterschiedlich beeinflußt In der vorliegenden Untersuchung konnte gezeigt werden daß nach Glutaraldehydfixation die parallelen Plasmamembranen in der Verzahnungszone der strialen und externen Sulcuszellen erhalten bleiben daß aber nach Osmiumfixation bestimmte Membranregionen offensichtlich in Vesikel aufgebrochen werden Diese Membranartefakte waren haufiger in den externen Sulcuszellen zu finden Als Grunde für die unterschiedlichen Fixationsergebnisse kommen ... auch die *verschiedenen Wirkungsweisen* mittel in Frage Unubliche Ultrastrukturen so... mit gewissen Vorbehalten interpretiert werd... vom Typ des verwendeten Fixationsmittels ... konnen

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COMMENTS ON THE ACOUSTIC-REFLEX RESPONSE FOR BONE-CONDUCTED SIGNALS

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Abstract Previous studies which have measured acoustic reflex responses to bone conducted signals have not effectively differentiated reflex responses from artifacts A convenient method for identifying such artifacts was developed and employed on some acoustic reflex meas ures for bone conducted signals The findings indicated that artifacts result when a frequently used acoustic ad mittance meter (Grason Stadler 1720B) and a conventional bone vibrator were used to measure reflex responses for tonal and noise activating signals It was suggested that the method be employed in future studies which investi gate the acoustic reflex in response to bone conducted signals

...t studies have suggested that the acous ...ref responds differentially for air- and ...one-conducted signals Djupesland, Flottorp, Sundby & Szalay (1973) reported acoustic-reflex thresholds for bone conducted tonal signals which were 5 to 25 dB lower in terms of hearing level than corresponding thresholds elicited by air-conducted tones Iwamoto & Pang-Ching (1975) reported a substantially greater percentage of acoustic-reflex re sponses to bone-conducted noise than to air conducted noise signals for both normal-hearing and hearing-impaired subjects Artifacts have been reported for acoustic reflex measures for high-level air-conducted signals (Danaher & Pickett, 1974, Niswander & Ruth, 1975, Margolis & Gilman, 1977) Since acoustic energy is generated in the external auditory canals during bone conduction stimulation (Tonndorf, 1966), similar artifacts may have occurred in the above studies Although the Djupesland et al (1973) study reported data from some control subjects which suggested that their results were not infl... by such artifacts, neither of the studies ...tively differentiated reflex responses ... facts for all of the data

The purpose of the present study ... obtain bone-conducted acoustic reflex ... ures employing a method which wa... signed to detect artifacts for each measu... procedure was developed which conve... ly measured the temporal characteristi... commonly used acoustic-impedance de... Results obtained with this procedure ... compared to the individual acoustic-r... measures for air- and bone conducted s... taken from normal-hearing and hearing... subjects, in order to separate artifacts f... true responses

Temporal Measurements of Acoustic-Impedance Devices

Several investigators have used an insta... neous change in a load impedance to mea... the temporal characteristics of acou... impedance measuring devices Su... Flottorp & Djupesland (1971) simulat... instantaneous impedance change by inse... an external probe signal into a known ... impedance This method required ... control of the external signal frequenc... internal access to the impedance appa... Margolis & Gilman (1977) simulated an ... dance change by altering the current ... driver circuit of the impedance device ... method eliminated the need for an ex...

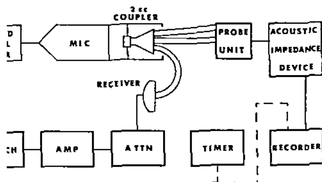


Fig 1 Block diagram of apparatus for determining temporal characteristics of acoustic impedance devices

source but still required internal access : impedance apparatus Morgan, Gilman ·ks (1977) produced a true change in the impedance by mechanically varying the ıe of a hard-walled cavity Although the ɔd did not require internal access to the lance device, it was somewhat restricted ; minimum response time and did not · for measurements of a resistive com- nt In addition to these specific problems, the methods required relatively sophisti l apparatus

ıe method used in this paper was based simulated electro-acoustic change in a impedance It employed apparatus found any laboratories and clinics and required iternal access to the acoustic impedance :e In addition, the method may be used etermine the temporal characteristics of : electro-acoustic impedance devices, re- less of the components the device meas or the probe frequency it uses

block diagram of the apparatus is shown 'ig 1 The probe unit of the acoustic edance device being measured was con- :ed to a standard 2 cc coupler (Bruel & r dB 0138) and microphone (Bruel & Kjær !) normally used for routine hearing aid ɔration The microphone output was fed in es to a sound level meter (Bruel & Kjær 3) an electronic switch (Grason Stadler 7B), an amplifier (Grason-Stadler 1288), an nuator (Grason-Stadler 1293), and a hear- aid receiver The output of the receiver was connected to the 2 cc coupler via the tubing on the probe unit which is normally used for varying air pressure during tympanometry With the electronic switch turned off, the static acoustic load impedance consisted of the combination of the acoustic impedances resulting from the 2 cc cavity, the coupler microphone, the hearing aid receiver and the associated tubing Activation of the electronic switch allowed the probe tone, generated by the acoustic-impedance device, to be amplified and directed back into the load impedance resulting in a simulated change in the static load impedance The steady-state magnitude of the simulated impedance change was governed by the gain of the feedback circuit and was measured directly with the impedance device Since the phase of the amplified signal was locked to the probe frequency at a con stant value governed by the apparatus, the change in the magnitude of the resistive component or the reactive component was deter mined by the magnitude of the total impedance change The time course of the simulated impedance change was governed by the rise and decay characteristics of the switch and a timer (Grason-Stadler 1216)

The output of the acoustic-impedance device was coupled to a complement of recording and analyzing devices consisting of an FM tape recorder (Precision Instruments PI 6200), signal averager (Nicolet 1010), and X-Y recorder (Hewlett Packard 7035B) However, any recording device, such as a storage

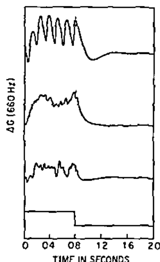


Fig 4 Change in acoustic conductance (ΔG) of the ear in response to 800 msec bone-conducted signals Top tracing 1 000 Hz Second tracing broad band noise Third tracing broad band noise in a subject with absent acoustic reflex by air conduction Bottom tracing temporal course of activating signal

sumed reflex threshold The first tracing represents the results obtained with a 1 000 Hz activator for a normal hearing subject The l characteristics of the tracing suggest the response was due to direct activation e measuring device Response latencies at onset and termination and response decay times were much less than those obtained with air-conduction signals, and were similar to the temporal characteristics of the device shown in Fig 2 Two additional characteristics of the tracing suggested that the response was an artifact First, the tracing shows a sinusoidal waveform during activation signal presentation along with a DC shift in the baseline value The number of cycles in this waveform changed in proportion to small changes in activation signal frequency Second, the substantial amount of overshoot at the termination of the signal was similar to the response of the measuring device These results suggested that the response was due to direct activation of the measuring device rather than true reflex activity

Similar results were obtained for the other normal-hearing subjects The mean "reflex" threshold determined from these aberrant responses was 75 dB re 1 dyne for a 1 000 tone Assuming that 0 dB HL for bone conduction at 1 000 Hz is approximately 24 dB dyne (Dirks & Kamm, 1975), mean "re threshold for the 1 000 Hz bone-cond tone was 51 dB HL This result was ide to the findings reported by Djupesl (1973)

The second tracing in Fig 4 shows re sponse of an individual subject to a bone ducted noise signal One third octave ba energy (centered at 400, 500, 630 800 1 000 Hz) were attenuated in order to lim energy in the area of the 660 Hz Again, the response latencies at the onse termination of the signal and the decay the response were considerably shorter for the air-conducted signals, and were s to those of the measuring device The form of the activating signal also appea be reflected in the response during presentation since the complex wavef the response disappeared when the signal terminated The response "decay may been related to a true reflex, but this c not be accurately determined because of presence of the artifact

In order to optimize the relationship tween the probe tone and the activating s additional one-third octave bands were s matically filtered from the noise-act signal and a lower probe tone frequency used Even when all components below Hz were attenuated, there was still evi of the presence of artifacts for all of the mal-hearing subjects when either the 220 F 660 Hz probe frequency were used

The mean "reflex' threshold by bone duction for the normal hearing subjects w dB re 1 dyne for broad band noise A comparison of these results to those fo conducted stimuli cannot be made sin frequency response characteristic of a b vibrator is quite different compared to th an earphone Nonetheless, the differen tween reflex thresholds for air-con tonal and noise-activating signals (P

is & Wiley, 1976) was not found when one conducted signals, again suggest-t the bone conduction responses were s

final check, reflex threshold measure were made on 3 hearing impaired sub ho represented a variety of pathologies resulted in absent acoustic reflexes bi-y for both air-conducted tones and and noise The third tracing in Fig 4 a response to a bone conducted band-noise signal in a 58 year-old female o measurable hearing in one ear and ate to severe sensori neural hearing loss other The temporal characteristics of icing and the presence of a complex orm during signal presentation again t that it was an artifact Similar results ound in a subject with a unilateral con e hearing loss and a subject with a uni-sensorineural hearing loss In all three g loss subjects a reflex like pattern hav e temporal characteristics of an artifact bserved in response to relatively low one conducted signals

DISCUSSION

findings suggest that the use of bone icted tonal and noise activating signals it the acoustic reflex results in artifacts using the Grason Stadler acoustic ad ice meter and a conventional bone vibra-Comparison of the temporal character-of the response to those of the device te that the responses were due to direct ition of the measurement device rather eflex responses

e reason that artifacts occurred in the nt study is not known One explanation be that the measuring device detected coustic energy generated within the ear s during bone conducted stimulation ilter response (Margolis & Gilman, 1977) e device used in this study may be broad gh to allow direct measurement of acous-iergy generated by the activating signal Although the previous studies used different equipment, this explanation may also account for their findings For example, the data of Djupesland et al (1973) showed that differences in reflex thresholds between air- and bone conducted signals tended to increase as the signal frequency approached the probe frequency This may be a reflection of the filter response of the measuring device Iwamoto & Pang Ching (1975) stated that "acoustic reflexes to bone conducted noise tended to be less well-defined than to air conducted noise' and that "bone-conducted noise stimulation resulted in slight balance meter oscillations regardless of stimulus intensity' which suggests that the waveform of the activating signal was measured directly by the apparatus

These data are not intended to imply that bone conducted stimuli will not elicit an acoustic reflex Because of the possibility of artifacts, however, future studies should take precautions in identifying artifacts during each measure, in addition to using appropriate control subjects Such precautions apply to clinical measures as well Methods which rely on visual observation of meter needle deflections, such as those used in the previous studies, are inadequate for identifying artifacts since the temporal differences between artifacts and reflex responses are quite small The method presented in this paper was intended to provide a procedure for accurate identification of artifacts *An alternative method would* be to employ non acoustic measures of middle ear muscle activity such as electromyography (Zakrisson, Borg & Blom, 1974)

ZUSAMMENFASSUNG

Vorhergehende Untersuchungen uber durch knochen

und auf einige knochengeleitete Mittelohrmuskelreflex messungen angewendet Die Ergebnisse zeigen daß Scheinreflexe vorkommen wenn man einen allgemein erhaltbaren akustischen Admittanzmeter (Grason Stadler

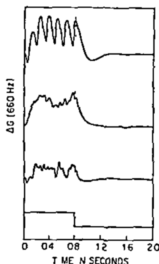


Fig 4 Change in acoustic conductance (Δ*G*) of the ear n response to 800 msec bone-conducted signals Top trac ing 1000 Hz Second tracing broad band noise Third tracing broad band noise in a subject w th absent acoustic reflex by air conduct on Bottom tracing temporal course of activating s gnal

sumed reflex threshold The first tracing rep resents the results obtained with a 1000 Hz activator for a normal hearing subject The ' characteristics of the tracing suggest the response was due to direct activation the measuring device Response latencies at signal onset and termination and response decay times were much less than those ob tained with air conduction signals and were similar to the temporal characteristics of the device shown in Fig 2 Two additional char acteristics of the tracing suggested that the response was an artifact First the tracing shows a sinusoidual waveform during activa tion signal presentation along with a DC shift in the baseline value The number of cycles in this waveform changed in proportion to small changes in activation signal frequency Sec ond the substantial amount of overshoot at the termination of the signal was similar to the response of the measuring device These results suggested that the response was due to direct activation of the measuring device rather than true reflex activity

Similar results were obtained for the other normal hearing subjects The mean reflex threshold determined from these aberrant re sponses was 75 dB re 1 dyne for a 1 tone Assuming that 0 dB HL for bone c tion at 1000 Hz is approximately 24 d dyne (Dirks & Kamm 1975) mean threshold for the 1000 Hz bone-con tone was 51 dB HL This result was id to the findings reported by Djupesland (1973)

The second tracing in Fig 4 shows sponse of an individual subject to a bon ducted noise signal One third octave b energy (centered at 400 500 630 8 1000 Hz) were attenuated in order to lim energy in the area of the 660 Hz Again the response latencies at the ons termination of the signal and the decay the response were considerably for the air conducted signals and were to those of the measuring device The form of the activating signal also appe be reflected in the response during presentation since the complex wavef the response disappeared when the sig terminated The response decay may been related to a true reflex but th s not be accurately determined because presence of the artifact

In order to optimize the relationsh tween the probe tone and the activating additional one third octave bands were matically filtered from the noise act signal and a lower probe tone frequen used Even when all components below Hz were attenuated there was still e of the presence of artifacts for all of the mal hearing subjects when either the 2 660 Hz probe frequency were used

The mean reflex threshold by bone duction for the normal hearing subjects dB re 1 dyne for broad band no se A comparison of these results to those f conducted stimuli cannot be made s frequency response characteristic of vibrator is quite different compared t an earphone Nonetheless the d ffere tween reflex thresholds for air-con tonal and noise activating signals (P

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FINE MORPHOLOGY OF THE ADVANCING FRONT OF CHOLESTEATOMA—EXPERIMENTAL AND HUMAN

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act The fine morphology of the advancing front : cholesteatoma and the mucocutaneous junction of .nted skin in the feline bulla have been investigated nmatory cell infiltration was frequently observed at dvancing front and the mucocutaneous junction It ggested that this frequent inflammation at this junc is due to the lack of a tight seal In the advancing epidermal migrating cells appear to be lower ous cells (or suprabasalar cells) rather than basal It appears that the slender cell processes of the ating cells make the initial attachment to the fibrin or basement membrane left behind by the de rated epithelial cells as a result of inflammation m grate along these structures using them as a guide lanted skin in the cat s bulla failed to develop a pearl iation but frequently developed an epiboly in which the epidermis receded and was partly replaced by ous membrane and its stroma was heavily invaded nucous epithelium resembl ng tubular glands

spite intense histopathological investigation aural cholesteatoma in humans (Schu echt 1974) and in experimental animals iedmann 1955, Ruedi, 1959 Fernandez & idsay 1960) the pathogenesis remains ntroversial The proposed theories are 1) ngenital cholesteatoma formation from ibryonic rests of squamous epithelium (Der ki & Clemis 1974) 2) squamous metaplasia mucosal cells to become keratinizing epi lium (Sade & Weismann 1977) and 3) migration of canal wall or tympanic mem ane epidermis by marginal perforation bramson et al 1977) from attic retraction ackingham 1968) or even by infiltration epidermal projections through the intact mbrane (Ruedi 1959 Fernandez & Lind y 1960)

Regardless of its origin, the keratinizing epi thelium has to grow, migrate and expand in order to form a cholesteatoma Therefore the modes by which migration of the choleste atoma (epidermis) occur in the middle ear be come an important research question It is critical to determine the factor(s) which main tain the balance between the epidermis and mucous membrane or which stimulate the epi dermis to migrate The purpose of this report is to attempt to clarify some of these questions through fine morphological investigation of the advancing front of cholesteatoma and muco-cutaneous junction of experimental animals

MATERIALS AND METHODS

Seven mongrel cats were prepared for study by implantation of full thickness canal skin into their middle ear spaces bilaterally via openings in the bullae A bed was prepared for each graft by scoring the mucosal surface The animals were then sacrificed at varying intervals 2 each were killed at one month 3 months and 6 months postoperatively The temporal bones were then serially sectioned and prepared for histopathological study The seventh cat was sacrificed at one month post-operatively and perfused intracardially with cacodylate buffered 3% glutaraldehyde and the area of skin and mucous membrane juxta position was dissected out and stored in the fixative overnight The specimens were then post fixed with *s* collidine buffered with 1%

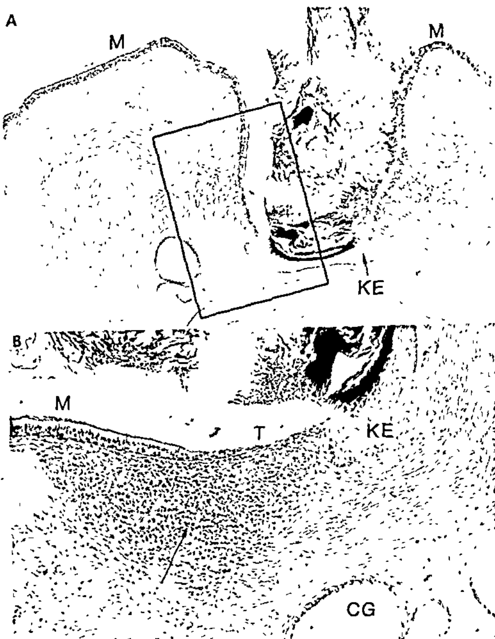
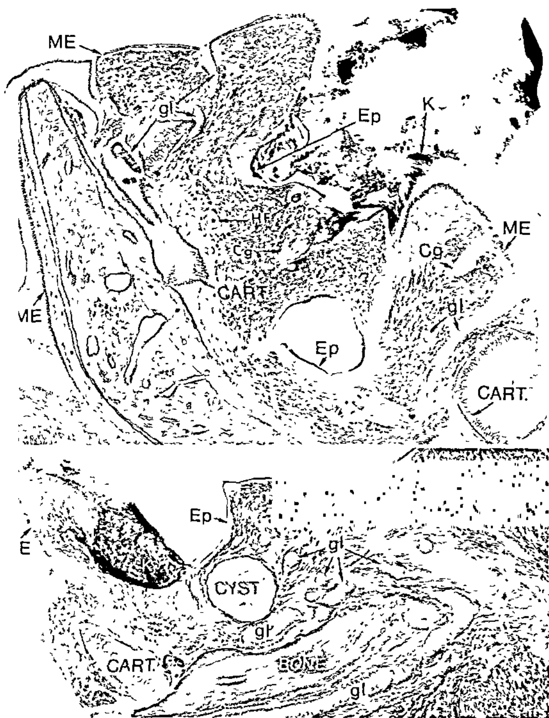


Fig 1 (A) A port on of ... shows ... mainde ... (M) C ... cell infiltration at the mucocutaneous junctions An area indicated by the rectangle is magnified in (B) H & E, ×65. (B) Higher magnification of mucocutaneou[s] ... from (A) shows heavy round cell infiltration ... Between the mucous epithelium (M) and the k[eratinizing] ... [epi]thelium (T ...

osmic acid for one hour and further processed for ultrastructural study, as described in an earlier report (Lim & Saunders, 1972).

Fresh specimens were obtained from 5 human subjects undergoing mastoid surgery. Patients were selected for study who had cholesteatoma matrix which demonstrated an advancing front making a confrontati[on] ... the mucuous membrane Specimens ... tained which would allow examinatio[n] ... junction between these two epithel[ia] ... and were processed for ultrastructur[al] ... as described above with the exceptio[n] ... fusion.



'2 (A) Implanted canal skin (*Ep*) in the cat bulla is ..ly taken over by the mucous epithelium (*ME*), which infiltrated deep into the implanted skin, forming 'ls (*gl*) Remnants of skin appendages, such as a hair ..le (*HF*) and ceruminous glands (*Cg*), are recogniz-. Cartilage (*Cart.*) of the external ear canal is rec-..able 6 months after the implantation *K* keratin H & E, ×30 (B) A close-up view of the same specimen as (A) (different section) shows cystic and glandular formation (*gl*) of mucous epithelium beneath the epidermis (*Ep*) from the original implant A portion of epidermis is replaced by mucous epithelium (*ME*) H & E, ×50.



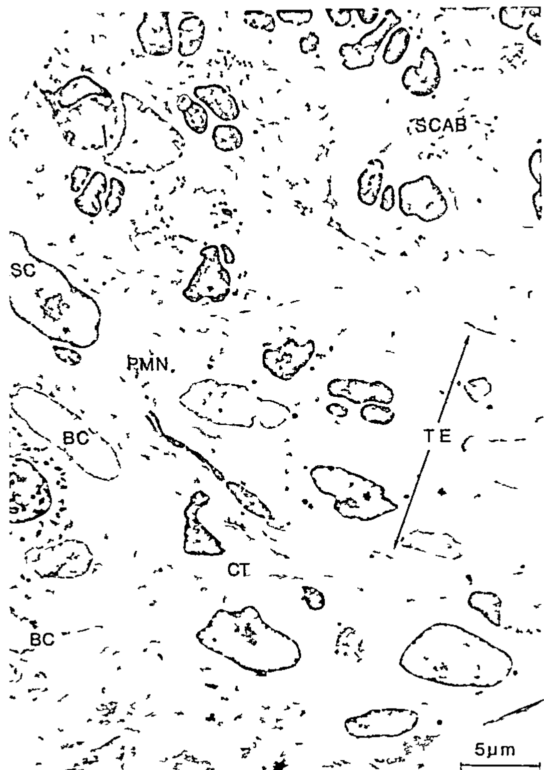
Fig 3 The inset is a light micrograph of mucocutaneous junction from a cat 22 days following skin implant in the bulla An epidermal (*KE*) spur formation is bordered by transitional epithelium (*TE*) which is covered by a scab (*S*) ×25 The area of the rectangle is shown in an electron micrograph which clearly demonstrates the ma- tween the epidermis and the transitional epith- and scab The epidermal spur is formed by ke granular (*G*) spinosal (*S*) and basal (*B*) layers. of the rectangle is shown in Fig 4

In addition, three serial-sectioned human temporal bones with cholesteatoma that were fixed with Heidenhain–Susa solution and embedded in celloidin were included in the study

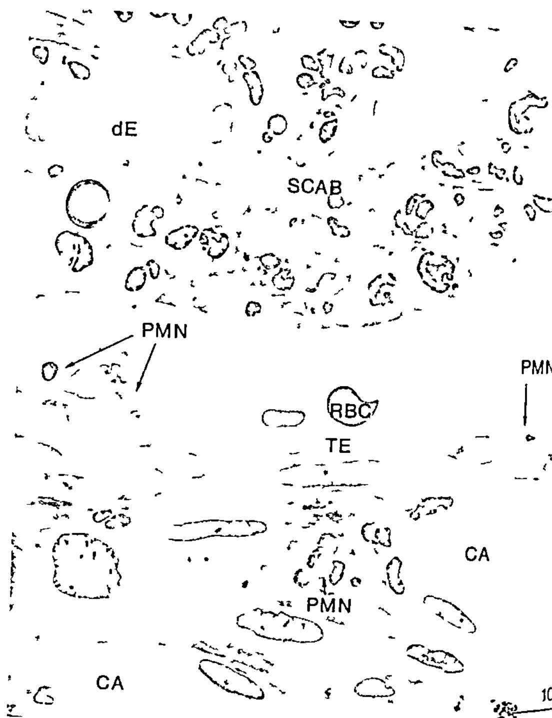
RESULTS

Animal materials

Four animals whose temporal bo- serially sectioned for study demonstra- viability of the implants, and in the re-



An electron micrograph shows the advanc ng front :p dermal spur A lower sp nosal cell (*LSC*) rather basal cell (*BS*) is mak ng d rect contact w th the trans tional ep thel um (*TE*) Numerous polymorphonuclear leukocytes (*PMN*) are inf ltrating the trans tional cell area *CT* connective tissue



F g 5 An electron m crograph of trans tional epithel um (*TE*) bordering the ep dermal spur shows non kerat n zing pseudostratif ed squamous type ep thel um The transi tional ep thel um evidently is covered by scab which is formed mainly of polymorphonuclear leukocy and degenerated ep thel al cells (*dE*) A crystal in the scab is interpreted as a cholesterol blood cell *CA* cap llary

F g 6 (A) A close up v ew of a basal trans t onal cell contain ng tonof laments (*TF*) ribosomes rough endoplasmic ret culum and a large oval nucleus Th s cell also possesses a few desmosomes (*d*) and hemidesmosomes (*arrows*) *CT* connective tissue (B) A close up v ew of F g 4 where a PMN made direct con what appear to be m grat ng ep thelial cells wh ch developed hem desmosomes w thout membrane *N* nucleus *CT* connecti e ti sue

G
d
TF
N
CT
d
1µm
N
ME
ME
PMN
CT
2µm

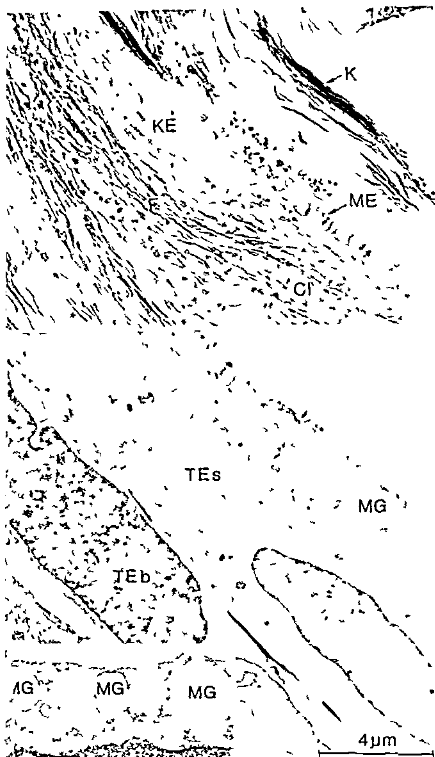
2 cats, which were excluded from this study, the epidermis was sloughed off None of the animals had tympanic membrane perforation at the time of sacrifice The implants consisted of typical stratified keratinizing squamous epithelium, made up of stratum basale, stratum spinosum, stratum granulosum and stratum corneum The implanted skin failed to develop a typical pearl, instead it formed an epiboly The great thickness of the connective tissue of the new mucous epithelium that encircled a patch of skin gave an impression of a cup, the bottom portion being the epidermis (Fig 1) A large portion of the epidermis of the implanted skin that formed the epiboly was replaced by a mucous membrane The epidermis may be receding rather than invading the mucosal membrane This interpretation is based on the fact that a thick dermis containing some epidermal appendages (such as sebaceous and cerumen glands and hair follicles) can be identified under the mucosal epithelium adjoining the epidermis (Fig 1) In addition, here was marked infiltration of mucosal epithelium resembling glandular structures inside implanted skin, further suggesting active igration of mucosal epithelium (Fig 2)

As expected, there was more evidence of acute inflammatory reaction in the animals that were sacrificed a few weeks after implantation than in those sacrificed later In the cat which was sacrificed 22 days after the implantation a scab consisting of necrotic debris and inflammatory cells was found overlying as well as inside the mucous membrane adjacent *to the mucocutaneous junction (Figs 3, 4 and 5)*

The epidermis at the junction was generally thickened by hyperplasia of the stratum malpighii when there was evidence of inflammation The hyperplasia resulted from increased cellularity, predominantly in the stratum spinosum (Fig 3) The edge of the epidermis (advancing front) resembles a wedge or plowshare, which appears to be identical with the "epithelial spur" in the healing of epidermal wounds (Croft & Tarin 1970) The most distal edge of the epithelium appears to undermine the mucous membrane (Fig 3) ... fact that the stratum malpighii had ... further into the junction than the granulosum and stratum corneum T... posed surface of the epidermal edge ... by flattened spinosal cells which pos... merous large cell processes It appears lower spinosal cells or suprabasal (Martinez 1972), rather than basal ... migrated to the raw surface of the co... tissue adjoining the mucosal epithel... 3 and 4) The migrating cells posses... cytoplasm, owing to the heavy accu... of the tonofilaments ribosomes and ... particles Although these cells d... hemidesmosomes where they made ... with the basement membrane (Fig 6... to cell desmosomes are only infr... seen *The migrating epithelial cells* ... made contact with the fibrin clots b... the cells are oriented and migrate ... appears that the migrating cells may ... old basement membrane that was le... by degenerated epithelial cells

The epithelial cells in the transition between the epidermal spur and ... front are also characterized by numer... processes, indicative of migration Th... propria underlying this area is invar... filtrated by inflammatory cells Poly... nuclear leukocytes (PMN) are a... mostly in the lamina propria imm... beneath the junction in cases where ... plant was made within a month In ... a PMN appears to have made con... *a migrating epithelial cell* giving an im... that PMN may be involved in epithelia... tion (Fig 6B) Lymphocyte and plas... infiltration became predominant in th... with a long history of implantation there is no remarkable round cell inf... at the junction the epidermal edg... thickened but still appears to under... mucous epithelium (Fig 7A)

The transitional epithelium closer ... mucosal epithelium became pseud... cuboidal epithelium or remained sq...



(A) Mucocutaneous junction between keratinizing ... lium (*KE*) and mucosal epithelium (*ME*) of a cat in ... skin was implanted into the bulla The epidermis ... rs to undermine the mucous epithelium or the ... us ep thel um migrated over the epidermis Note that ... a few round cells have inf ltrated (*CI*) K keratin *F* collagen fibers ×400 (B) A superficially located transi tional epithelial cell (*TEs*) contains secretory granules (*MG*) whereas the basally located transitional epithelial cell (*TEb*) conta ns tonofilaments An inset shows mem brane bound secretory granules (*MG*)

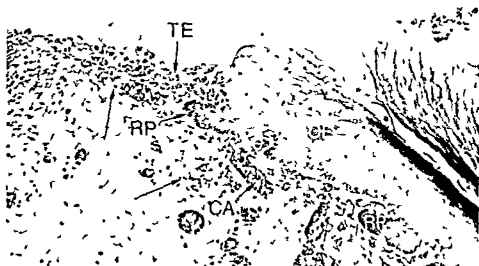


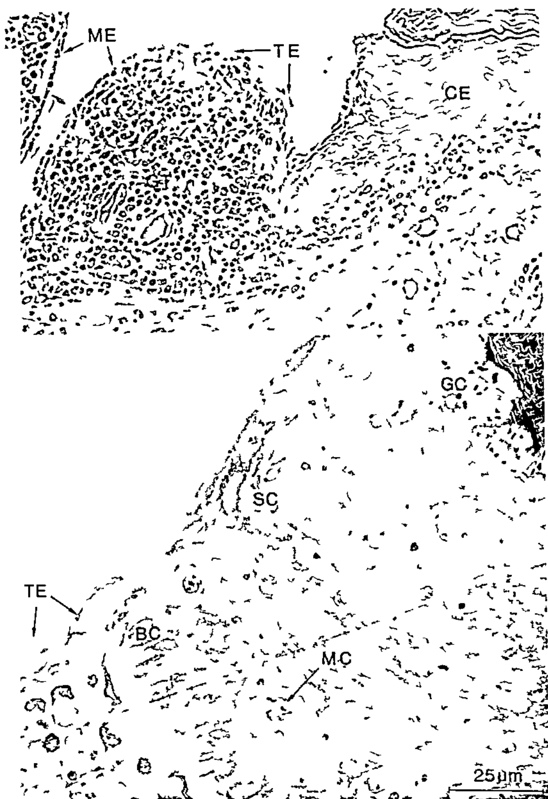
Fıg 8 A human temporal bone of a patıent wıth chol esteatoma shows that the advancıng front of choleste atoma (*CE*) forms an epıdermal spur facıng the transı tıonal epıthelıum (*TE*) Observe engorged blo (*CA*) and exaggerated rete peg (*RP*) and hea tory cell ınfıltratıon at the junctıon A keratı

type These transıtıonal epıthelıal cells possess characterıstıcs of metaplastıc epı thelıal cells, contaınıng tonofılaments ın the basal cells (Fıg 7B) and secretory granules ın the superfıcıal cells (Fıg 7B) Even ıf these cells contaın only a few secretory granules, theır Golgı apparatus are well developed, ındı catıng the potentıal of secretory actıvıtıes by these cells (Fıg 9) The transıtıonal cells gradually became typıcal mucosal epıthelıum

Human materıals

The basıc morphologıcal fındıngs are essen tıally ıdentıcal wıth those of the anımals (Fıg 8) except that there are no skın appendages such as were seen ın experımental ımplants The ınvagınatıon of the rete peg ıs more pronounced ın human specımens (Fıg 8) The epıdermal advancıng fronts are also thıckened by hyperplasıa of the malpıghıan layer The spınous layer contaıns about twıce as many cells (12 cells thıck) as the adjacent area (5 cells thıck) (Fıg 9 A) Some spınosa are found near the basal cells show e cell dıvısıon (Fıg 9B), ındıcatıng proliferatıon at thıs sıte The exposı of the advancıng front ıs covered squamous type spınosal cells whıch acterızed by dark cytoplasm and cell processes on the free surface faces of the spınosal cells facıng the cells are well covered by desmosom occasıon several secretory granule-

Fıg 9 (A) A phase contrast mıcrograph specımen from a patıent wıth cholestea dermıs shows the advancıng front facıng the tıssue (*GT*) covered wıth transıtıonal epıthel mucous epıthelıum (*ME*) ×250 (B) An ele graph of the same specımen as (A) (dıffer demonstrates the face of an advancıng front up of keratın (*K*) granular cell (*GC*) spınos and basal cell (*BC*) layers A mıtotıc cell (*M* the spınosal cell layer The adjoınıng conne covered by transıtıonal epıthelıum (*TE*) whıc be ın contact wıth a spınosal cell


ME
TE
CE
GC
SC
TE
BC
MC
25 µm

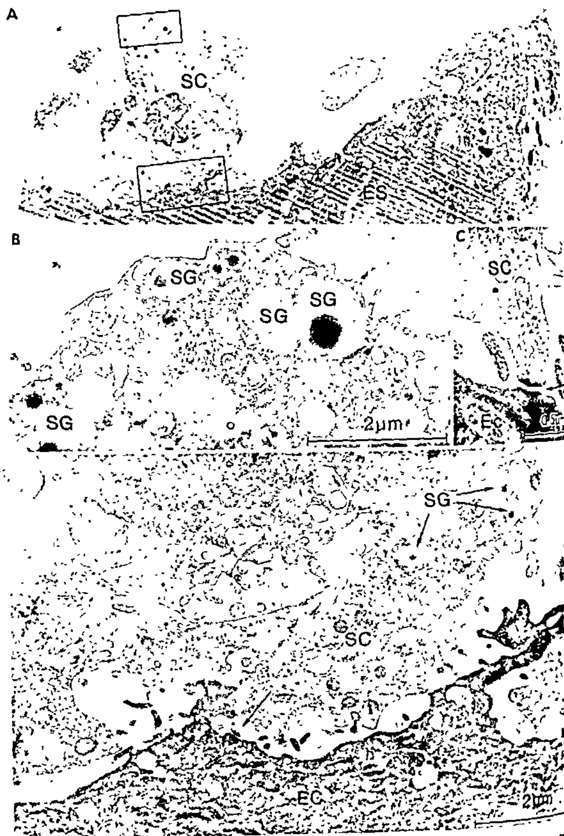


Fig 10. (A) Several secretory cells (*SC*) are attached to the spinosal cells of the epidermal spur (*ES*) Upper rectangle is enlarged in (A) and lower rectangle is enlarged in (D). (B) Close-up view of the secretory granules (*SG*) from (A) (C) Close-up view of the desmosome (*arrow*) between a spinosal cell (*EC*) and a secretory c (D) Desmosomal attachment (*arrow*) between a cell containing secretory granules (*SG*) and a sp with dark cytoplasm (*EC*)

ere observed to be attached to the free : of the advancing front by desmosomes)) Since we were not able to find secre- ‧lls in the vicinity of the epidermal spur ould be accounted for as a possible of contamination, we have tentatively d that these secretory cells did not come he neighboring mucous epithelium and iot ruled out the possibility that these ory cells could have originated from the mis The basal cells also appear thick- lue to the tall columnar arrangement at lvancing edge As in the animals, lower sal (or suprabasalar) rather than basal are the ones that give the appearance ive migration due to their large pseudo- and the absence of desmosomes

: mucocutaneous junction again are ly infiltrated by round cells and PMN, animals The most striking finding in ns, however, is heavy infiltration of ia cells, lymphocytes, and macrophages ie submucosal connective tissue (or ilation tissue) adjoining the epidermal iting front (Fig 11)

e mucous membrane side of the muco- ieous junction is again characterized by uboidal type or simple or stratified squa- , type of transitional epithelium (Fig , as in the animals, until it becomes a al mucous epithelium with goblet cells ciliated cells These cells are charac :d by abundant tonofilaments, glycogen :les and/or ribosomes, and numerous cell :sses (Fig 11B) Some cells contain dark plasmic inclusions (lysosomes), as de ed by Martinez (1972) As in the animals, nigrating squamous cells in human speci s are closely related to fibrin clots and/or ment membrane left behind by degener- epithelial cells It appears that migrating first send their slender cell processes to h to the fibrin clots or basement mem e (Fig 12A), and then the whole cell es move along using these structures as es or anchors (Fig 12B) No ciliated cells : observed in this transitional epithelium

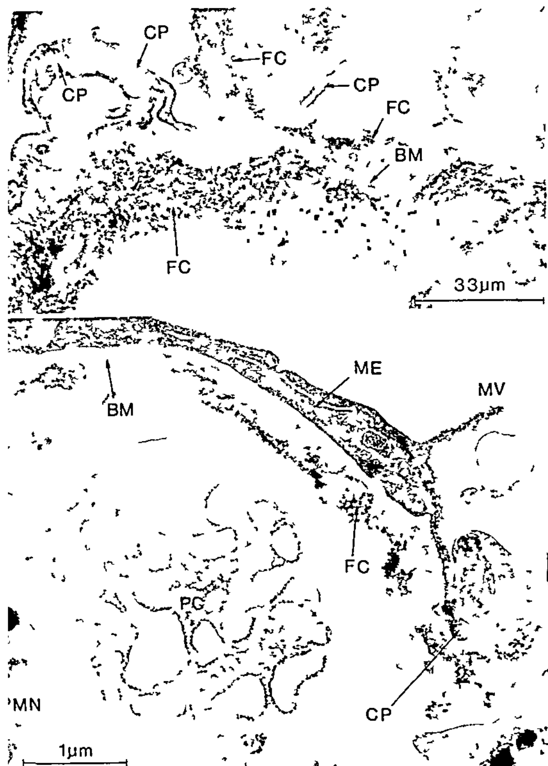
Hemidesmosomes were frequently seen in the transitional epithelial cells, but junctional complexes were absent

DISCUSSION

The first question that we entertained in this study was regarding the behavior of the migrating epidermis and mucosal epithelium when they confront each other in the middle ear cavity It has been established that the interaction between two epithelial cell types is governed by the phenomenon of "contact inhibition" This concept is illustrated by a classical experiment by Weiss (1959) Using phase contrast time lapse photography, he observed the random migration of epithelial cells in tissue culture When two different cell types were combined, they tended to aggregate into colonies containing only cells of the same type When two like cells collided, they drew closer together and remained joined When two unlike cells met, however, they drew away from each other and continued their random locomotion It was the ability of the cell to recognize sameness or foreignness through contact which determined its behavior Chiakulas (1952) also made such an observation by grafting various epithelial types to wounds made on amphibian embryos He found that when the graft was of epidermis or an epithelial type normally found adjacent to skin, e g , oral mucosa or cornea the migrating fronts of epidermis would meet and fuse with the edges of the graft and quickly resume a steady state When foreign epithelium, e g , gall bladder or lung, was grafted, however, the advancing front of squamous epithelium would migrate over or under the graft until it met and fused with the opposite skin edge It has not been clearly established, however, whether the mucosal epithelium of the middle ear recognizes the implanted epidermal epithelium as similar or dissimilar Since canal wall skin does not normally reside adjacent to middle ear mucosa, it can be suggested that the implanted epidermal epithe



Fig 11 (A) A portion of granulation tissue adjoining the advancing front of cholesteatoma is covered by transitional epithelium (*TE*), and the stroma is infiltrated by numerous plasma cells (*P*) and a few lymphocytes *CA* capillary; *CT:* connective tissue. (B) A transitional cell covering the granulation shows dark cytopla[...] dense ribosomes and abundant tonofilaments [...] are very few desmosomes, but these cells p[...]merous large microvilli (*MV*) *BM* basement m[...] *HD* hemidesmosomes, *CT* connective tissue



12 (A) A close up v ew of the advancing front of steatoma where the port on of a long cell process of a migrat ng ep thel al cell made contact with ants of basement membrane (*BM*) by f brin clots (*FC*) (B) A m grating ep thel um (*ME*) has made contact with f brin clots (*FC*) and oriented its d rection of m gra t on *MV* microv ll *CP* cytoplasmic process *PMN* polymorphonuclear leukocytes

lium and middle ear mucosal epithelium would, upon contact with each other, perceive each other as dissimilar and remain separated

They also may perceive each other as similar for the following reason Experimental studies have shown that the type of underlying mesenchyme can determine the differentiation, rate of growth, and spread of embryonal epidermis (McLoughlin, 1968) It has been further demonstrated that the specificity of adult epidermal transplants is dictated by the recipient dermis (Billingham & Silvers, 1968) The fact that our implanted skin surface was partly replaced by the mucosal epithelium suggests that the connective tissue alone does not dictate the type of epithelium to be supported Our experiment appears to support the concept that the dermis of the ear canal can also support the middle ear mucosal epithelium or vice versa Because the keratinizing epithelium and mucosal epithelium can be derived from the same stem cells, they may recognize each other as similar cells (Peck, 77, Sade & Weismann, 1977) The attach- of secretory cells to epidermal cells in esent study may support the idea that the etory cells may have derived from the keratinizing epithelium, assuming that these cells had not been accidentally implanted If this is true, then the mucosal epithelium and keratinizing epithelium should be compatible and fuse together to form a tight seal The present findings, in general, do not support the evidence of a tight seal, as the mucocutaneous junctions are frequent sites of inflammation and/or infection Even if they recognize each other as similar, and presumably form a seal, our study suggests that this is a very weak mucocutaneous junction They appear to confront each other with the two epithelia competing for space in the middle ear rather than stabilizing their relationship

It has been observed on many occasions, both clinically and experimentally, that ag gressive behavior of cholesteatoma is invariably associated wi ction and inflammation (Ojala & Sax Walsh et al, 1951, Rüedi, 1959, 1963, Fernandez & say, 1960, Harris, 1962, Schechter 19 & Saunders, 1972, Abramson et al 1977, Lim et al, 1977) Rüedi (195 talc as an inflammatory stimulus to the surface of the tympanic membrane of pigs, demonstrated that the inflam seemed to stimulate basal cell prol and penetration of the lamina propria intact pars flaccida Fernandez & L (1960) noted that inflammation p hyperplasia of the tympanic membra dermis to ten to fifteen times normal ness The present investigation suppor observation in that the proliferation dermis is stimulated by inflammation

Actual migration of epithelium how independent from proliferation and h shown in healing wounds to be u by addition of mitotic inhibitors (Kr 1972) The influence of inflammation ever, also seem to govern the migr epithelium by the concept of conta ance' Weiss (1959) first described the nomenon when he observed that co tissue cells would elongate, align selves with the long axis of parallel co fibers on the undersurface of fish sca cells would migrate only parallel w fibers indicating that the migration of cells was regulated by the fibers The ing fronts of epithelia of healing wou also regulated by the contact guidance (Giacometti & Parakkal, 1969) It h shown that in the initial stages of wou ing an inflammatory exudate bridges th The exudate contains a fibrin net w the phenomenon of clot retraction orient the fibers so that the major parallel from wound edge to wound Thus, they form a guidance system orients migrating epithelial cells to th edges t (Baier, 1972) It was confi ltrastructural study of healing ithelial cells at the front al ves parallel to fibr at the e and seem

them (Martinez, 1972) We observed an cal situation at the point of confronta between cholesteatoma and middle ear sa There is an advancing front of kera- ig squamous epithelium and an inflam- y zone ahead of it We have confirmed nigrating epithelial cells align themselves the fibrin net from the transudate caused flammation Furthermore, we have also rmed the earlier finding that the old base- membranes left behind by degenerated as a result of inflammation play an im nt role in new epithelial migration (Lim , 1977)

e above findings, then, are supportive of iotion that the presence of inflammation : *sine qua non* associated with choleste- a invasiveness, as has already been sug- d (Lim & Saunders 1972, Lim et al ,) Inflammation dictates this behavior, by stimulating proliferation of epidermis by forming guidance pathways directing igration It is the inflamed stroma, or peri- ix which determines the extent of the sive nature of a cholesteatoma rather than nherent characteristic of the squamous ielium of cholesteatoma itself The reason the frequent inflammation present at the tion in the absence of gross middle ear tion remains undetermined It may be the lt of microorganisms which have gained ss to the subepithelial connective tissue in w area or epithelial gap at the junction, hich case there may be a separation of the epithelia due to the lack of a tight seal junctional complex However, we cannot out the possibility of an immunological

iu i

he origin of the transitional epithelial cells id between the cutaneomucosal junction ld not be determined with certainty in the sent study However, we suggest that these s, at least in animals, are migrating muco- cells rather than epidermal cells on the owing grounds First, the implanted epi- dermis generally receded if kept over a long period (2 months or longer) and were replaced by mucosal epithelium Second, the transitional epithelium very closely resembles the metaplastic mucosal epithelium (non keratinizing) found in the human middle ear mucosa described earlier (Lim et al , 1977) Third, these cells contain secretory granules On the other hand, in human specimens, where the cholesteatoma is expected to spread rather than recede, some transitional cells close to the epidermal spur may well be migrating epidermal cells This study could not resolve the question of what factors dictate which epithelium takes over or is transformed into the other It can be suggested that one of the factors could be the gaseous environment, such as the content of CO_2 versus O_2, and/or humidity (Sade & Weismann, 1977) It is also possible that the basal cells of the transitional epithelium could derive from epidermal cells and the superficial cells from mucous epithelium and that the eventual survivors may be determined by the environmental factors Further controlled experiments are required to answer this question

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ZUSAMMENFASSUNG

Es wurden die Ultrastruktur der vorruckenden Grenzzone des Cholesteatoms und der mukokutanen Ubergangszone von in die Bulla von Katzen implantierter Gehörgangshaut untersucht Sowohl in der vorruckenden Grenzzone des

Wanderzellen eher niedere Stachelzellen (oder supra

basale Zellen) denn Basalzellen zu sein Es scheint ferner daß die schlanken Zellfortsatze der Wanderzellen den ersten Kontakt zu Fibringerinnseln und/oder zu Basalmembranresten herstellen die von als Folge von Entzundung degenerierten Epithelzellen zuruckgelassen wurden und daß sich die Wanderzellen dann entlang dieser Strukturen fortbewegen sie als Leitlinien benutzend In die Bulla von Katzen implantierte Haut bildete keine Epithelperlen sondern entwickelte sich haufig ahnlich wie ein Keim im Stadium der Epibolie In diesen Fallen zog sich die Epidermis zuruck und wurde teilweise durch Schleimhaut ersetzt *wahrend in ihr Stroma schleimbildendes Epithel* nach Art tubularer Drusen eindrang

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A COMPUTER CONTROLLED BINAURAL BALANCE TEST

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(Received October 10 1977)

t The commonly used ABLB procedure (Alter
naural Loudness Balance) is strongly influenced
psychological factors the consistency of the re
ide and the increase in intensity in equal stages on
e To avoid this influence side and intensity have
andomized The test has therefore been subjected
iputer control and the results are continuously
d as to their significance 26 patients with sus
inner ear disturbance showed a significant relative
ment by contrast in not one of 14 normal hearing
is was the result positive This test takes no longer
e conventional procedure

is still much controversy in the litera concerning the side of reference when rming the ABLB (Alternate Binaural ness Balance), (Priede & Coles, 1974, l 1977) Neither of the commonly prac procedures of this test—the method de ed by Jerger and that of Hood (Reker, a)—is in keeping with modern principles sting, in order to avoid any psychological nce a randomization of the reference neter is required (Sachs 1974) Such hological effects are provoked by the fact the intensity is fixed on the same ear ighout the entire test and that the incre s are identical and thus constitute a e of error This phenomenon can be dem rated when testing normal hearing in luals in whom the performance of a bal- test must also be possible As mentioned w 14 normal persons were tested, two al results are shown in Fig 1

avoid such errors, the choice of the side ell as of the intensity must be governed random selection The most favourable edure is to apply pseudorandom numbers g a computer on line, the machine can monitor the significance of the results through out the entire testing procedure, and is thus in effect in control of the test

As described by Priede & Coles (1974) the points of equal loudness are not situated exactly in a straight line but form a gentle curve The difference between the curve and the line (Fig 2*a*), which is drawn as a simplification, is so small that it cannot be proved with that number of values which one usually gets, it is essential, however, to limit the range of testing to about 25 dB above the threshold of the worse ear, as beyond this range the difference between the curve and the line widens

By correlating all pairs of values the regres sion line is found, represented by the equation $y=bx+a$ ' b' is the tangent of α and thus represents the angle (Fig 2*b*)

The aim of this procedure is to determine the significance of the deviation of the angle from 45° (i e the deviation of b from 1) To achieve this, the standard deviation of b ("s_b") is needed (Sachs, 1974) With the formula shown in Fig 2c one is able to obtain the *T*-value, which represents the significance (Student distribution) of the deviation of the angle, caused by the relative recruitment

As mentioned above, the whole procedure is computer controlled (Hewlett Packard 2100A) First the machine requires inputs Other than data such as test number and date, both the thresholds and the maximum intensities must be stated, up to which the test can be carried out on the right, resp the left ear The computer then selects one of the two possible sides according to random numbers and an intensity which represents a randomized

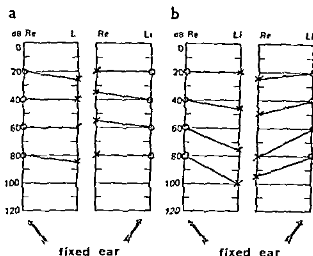


Fig 1 Balance test in normal individuals two typical results (*a*) As it would be expected and often occurs (*b*) can also be seen frequently

value in the existing gap (this value also corresponds to a simulated Gaussian distribution) The audiometrician sets the audiometer to this value (in Fig 3*a* it is 54 dB for the right ear) The patient sits beside the audiometer (without being able to see the potentiometer) he himself alters the intensity presented to contralateral ear to the value (44 dB) which considers to be equivalent (a Peters audiometer AP6 was used, which can give a sweep intensity, alternate tones), in no way is the pa-

tient influenced by the audiom... value is fed into the computer wh... the side and intensity for the next ... 3*a*, 56 dB to the right ear) Again ... searches for the corresponding loudn... other ear and this procedure can be ... as often as wanted—in Fig 3*a* 11 tim... ly 10–15 pairs of values have been ... the patient is searching for the corr... loudness the computer prints out ... significance, the deviation of the a... 45° and the side of the relative re... The procedure is concluded by a spe... (1E10) Finally the computer prese... statistical data and draws the d... 3*b*)

RESULTS AND DISCUSSIO...

Using the test procedure described ... hearing persons were examined none ... any significant recruitment (the limit ... cance was 5%) In addition both the ... procedures were used on these subje... testing the right ear, the medium ... was 0 978, on the left ear it was ... difference is significant (1% U test ... This shows that the result is depend... side on which the tone was fixed (bec...

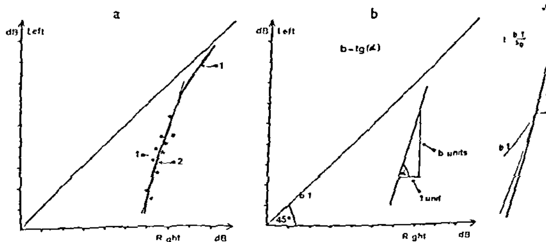


Fig 2 The principle of the test procedure (*a*) A line is drawn through the points which represent the pairs of values (?) This line is a negligible simplification in comparison with the curve (1) in the range 20–30 dB above threshold (*b*) The value of "*b*" represen... (*c*) The calculation of the standard deviati... with help of the formula shown the calcul... significance is possible

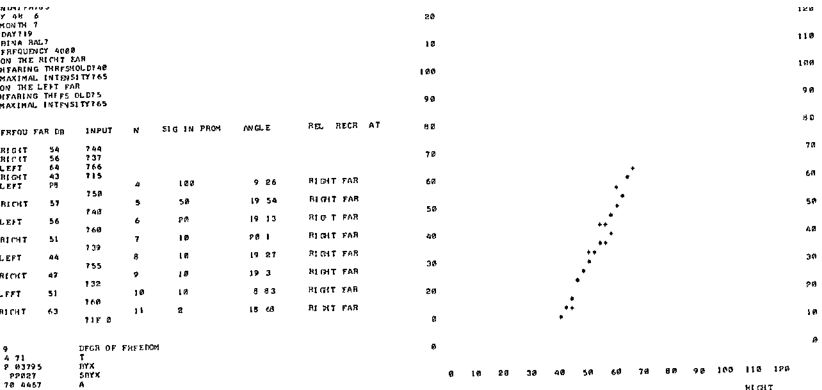


Fig 3 Example of a printout (*a*) First several questions are asked by the program a heading is printed and then the computer makes a random choice of side decides the intensity and waits for an input (query mark) This procedure is repeated as often as the audiometrician wants—it is ended by the input 1E10 While the patient is searching for equal loudness at the other ear statistic data are printed out (the significance of the angle and the side of the relative recruitment) (*b*) Further statistical data are then printed out (the degrees of freedom the *T* value of the Student distribution *b* and *a* of the equation $y=bx+a$ and the standard deviation of *b* the s_{bxx}) and a diagram is printed on which the pairs of values are represented *by crosses and the regression line is given by stars*

is a comparison of two methods both applied on the same person, this result cannot be affected by subclinical hearing disorders) It must be suspected that this psychological factor affects the result in the same way when testing persons with hearing disorders

Twenty-six patients with a unilateral sensorineural hearing loss were also tested This group of patients had been investigated with other audiological procedures, calorization and X-ray According to the anamnesis the cause of the hearing disturbance was M Meniere, vertebrobasilar insufficiency (showing no retrocochlear symptoms) or unilateral *noise-induced permanent threshold shift* It may be assumed that in all or almost all of these cases the disturbance was located in the inner ear

The randomized test demonstrated a significant recruitment in all 26 cases and the mean value of the deviation of the angle—the difference between the regression line and the diagonal—was 14 87° (s = 5 96°) In these cases the test procedures according to Jerger and Hood had also been performed Hood's method revealed complete recruitment in 23, and Jerger's method in 8 out of the 26 cases

These results prove that the randomized test procedure with the calculation of significance is a sensitive examination procedure there was a positive relative recruitment in all patients with inner ear disturbance The test does not take any longer than the conventional procedure, its one disadvantage is the necessity of a calculator, though a machine much smaller than the one used would suffice (a portable programmable calculator such as the Hewlett-Packard 97)

Apart from using this testing method—which calculates the beginning of the curve as a straight line—it should also be possible to calculate the entire testable range as a curve This procedure may offer further diagnostic possibilities, but a considerably greater number of pairs of values would be necessary for the calculation of a curve with s[illegible] curacy

ZUSAMMENFASSUNG

Die ubliche Durchführung des audiol[illegible]

die Zwischenergebnisse immer wieder au[illegible] uberpruft 26 Patienten mit vermutlichem In[illegible] den hatten bei dieser Testdurchführung ein [illegible] relatives Recruitment im Gegensatz dazu [illegible] von 14 Ohrgesunden das Ergebnis pos [illegible] verfahren dauert nicht langer als die herk[illegible] fuhrung

RÉSUMÉ

D habitude la procedure de « ABLB » ([illegible] binaural loudness balance ») est influencée [illegible] teurs au niveau central Premièrement l'[illegible] ference ne change pas et deuxièmement l'[illegible] d intensité du son est effectuée en incré[illegible] Nous presentons une modification nouvelle [illegible]

teste Chez 26 malades avec une lésion coch[illegible] trouve un recruitment significant Cependant [illegible] sonnes avec des oreilles internes normales [illegible] présenté un resultat qui était significant pour [illegible] ment Ce teste modifié ne dure pas plus [illegible] version utilisée habituellement

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SONOTUBOMETRY

An Acoustical Method for Objective Measurement of Auditory Tubal Opening

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ct The sound conduction method for measurement Eustachian tube opening has been studied and cations made in the test procedure to increase its erformance and reliability By holding the sound near to the nostril mounting the microphone circumaural ear defender and by feeding the output microphone through a filter with a very narrow idth (3 16 Hz) many of the pitfalls of the sound ction technique described earlier could be removed ding of the transfer function between the nasal tip xternal auditory meatus and recording of the spec of swallowing sound revealed that the useful fre y range for the measurements was upwards from Hz By using the three frequencies 6 7 and 8 kHz ethod showed tubal opening in 90 or 95% of normal depending on whether the minimum amplitude ited was ≥5 or 1–4 dB respectively

inical practice, testing of the Eustachian function is performed with a wide variety iethods Simple tests for anatomical pa y alone are not adequate and should complemented with more sophisticated iods Much progress has been made in the ·lopment of tests for assessment of middle pressure and ventilatory function How- , there is still a need for simple objective iods in the evaluation of physiological tory tubal opening One of the earliest blished methods used for this purpose is ieasure how sound is conducted through Eustachian tube (Politzer, 1869, Perlman, 1951)

he sound source in this method was lo- d some distance from the subject and the sound was transmitted through a rubber into the nasal cavity Perlman (1951) pointed out that the length of the tubing connecting the ear to the microphone affected the recorded response because of resonances This phenomenon was investigated by changing the length of the coupling tube and as a solution to some of these problems the lengths of the tube were selected so as to conform to the wavelength of the test sound used (Naunton & Galluser, 1967) The leakage of sound to the microphone directly through the connecting tube or from the sound source interfered with the evaluation of tube openings Consequently Naunton & Galluser (1967) mounted the microphone in a 1½ inch (38 mm) thick wooden box, thus preventing any direct sound transfer into the microphone from the sound source There were also pitfalls of possible sound leakage from the external meatus and of noise introduction into the system, if the ear plug did not give adequate seal

Satoh et al (1970) attached the microphone tightly to the external ear canal and the speaker hermetically to the nostril by wrapping the adapter with adhesive tape Eguchi (1975) abandoned the use of two tubings and instead directly inserted a hearing aid earphone as a microphone to the external meatus, and another earphone as a sound source to the orifice of tubal catheter

The frequencies of the external sound source have ranged from 60 to 2 000 Hz (Table I) Perlman (1951) stated that a tone of 100 Hz is more satisfactory for use than one of higher frequency Tukamoto (1957) stated that any

Table I *The frequencies of delivered sound according to several authors*

Author	Year of *publication*	Frequency *(Hz)*
Perlman	1939	500
	1943	60
	1951	*100*
Tukamoto	1957	1 024
Elpern et al	1964	200
Naunton & Galluser	1967	200
Flach & Seidel	1967	1 000
Satoh et al	1970	1 930
Cole and Cole	1974	200
Eguchi	1975	2 000
Ogawa et al	1976	2 000

frequency could be used as a test sound He employed the test tone of 1 024 Hz because it best suited his sound source According to Elpern et al (1964) the test frequency of 200 Hz chosen caused with their transducer less acoustic radiation outside the head than the higher frequencies and gave an additional advantage of a low attenuation in narrow tubes *Flach & Seidel (1967)* used a loud[illegible]aker as a microphone and a test tone of [illegible] Hz, on the grounds of the microphone's [illegible]cteristics On the other hand Naunton & [illegible]alluser (1967) regarded a 200 Hz sound as a good compromise between sound leakage and efficient sound conduction in their "E T Analyzer" The features of the apparatus devised by Satoh et al (1970) also decided the frequency of delivered sound

As a rule the microphones used were sufficiently good to detect the test sound at the external canal and generally the studies were carried out with the use of a calibrated condenser microphone The microphone was connected to an amplifier and the output of an amplifier was earlier recorded directly with an oscillograph (Perlman, 1939, 1951, Tukamoto, 1957), and later also with a pen recorder (Elpern et al , 1964 Naunton & Galluser, 1967, Satoh et al , 1970, Eguchi, 1975)

Although all the testing was done in a sound proofed room there was a possibility that the microphone might pick up some ambient noise Elpern et al (1964) first filtered [illegible] put of an amplifier (Bruel & Kjær Ty[illegible] with a filter set (Bruel & Kjær Ty[illegible] *using a third octave filter* with a ce[illegible]quency of 200 Hz The −3 7 dB ban[illegible] of this type of ⅓ octave filter, also se[illegible] *Flach & Seidel (1967)*, at a centre fre[illegible] of 200 Hz is approximately 46 Hz a[illegible] to IEC Publ 225–1966 (Bruel & Kjær [illegible] catalogue 1974) The 'E T Analyzer' [illegible] by Naunton & Galluser (1967) and h[illegible] measuring frequency 200 Hz only had [illegible] pass filter to filter out the transients and disturbing frequencies outside of th[illegible] *band It had a flat response between* [illegible] 205 Hz, its high and low pass rolloff [illegible] 35 dB/octave The filter of the appar[illegible]signated by Satoh et al (1970) rejected [illegible]quencies below 1 500 Hz and above 2[illegible] prevent disturbances The filters hithe[illegible] have had a relatively wide bandwidth [illegible] disturbing noise could influence the [illegible] response

Because of inaccuracies in the meas[illegible] technique itself (the ear moulds w[illegible] placed exactly in the same position [illegible] time), the resonance and antires[illegible] effects due to the variations in the size [illegible] and oral cavity, nasopharynx and phar[illegible] because of effects of nasal abnormalities as deviation of the septum etc the[illegible] large fluctuations of the "normal res[illegible] difficulties in interpreting them (Ta[illegible] Considering these data, which are [illegible] complete and considering the accep[illegible] this type of method into clinical use th[illegible]ent study was carried out in order to [illegible] investigate the *sound conduction* test.

EQUIPMENT

The equipment consisted of an insert ea[illegible] as a sound source, a microphone [illegible] into the circumaural ear defender, a fre[illegible] analyser functioning as the signal sou[illegible] amplifier and as the filter and a grap[illegible] recorder (Fig 1) *The frequency analyse*[illegible]

II *The recorded tubal openings on normal people in different materials*

t	Number of Eustachian tubes	Pos tive response (%)	No positive response	Sometimes positive response
ito	40	3? (80)	8	–
n & Galluser	15	8 (53)	2	5
Se del	161	109 (68)	52	–
t al	31	23 (74)	8ª	–
Cole	28	25 (89)	1	2

ªormal find ngs

rodyne Analyzer, Bruel & Kjær type consists of an analyser section and a beat ncy oscillator section The output signal the oscillator is fed into the insert ear (Hearing Aid Earphone Oticon A/S AF M8) connected to a changeable olive tip held by the patient snugly in f his nostrils It was necessary in order to n a satisfactory signal to noise ratio for ound pressure level of the earphone to from 100 to 112 dB (re 20 μPa) as meas with the sound level meter (Bruel & Kjær 2209) in combination with an octave set (Bruel & Kjær Type 1613) using a ³ coupler (IEC Recommendation R 126– The possible pressure changes in the cavity do not influence the sensitivity of earphone during swallowing and its fre cy response curve was nearly straight in ange of 100 to 2 000 Hz with deviations arger than 3 dB (Virtanen 1977)

e test sound reaching the external audi meatus through the Eustachian tube was d up by the calibrated condenser micro- e (Bruel & Kjær type 4134) connected to preamplifier (Bruel & Kjær type 2619) was embedded into the commercially able circumaural ear defender (Exel OY ta Super) (Fig 2) The tested shielding t of the ear defender before mounting on an average 40 dB for frequencies in the of 2 to 8 kHz (Physikalisch Technische lenanstalt Germany 1971) In the wall of ar defender a thick plastic window and e the ear defender a small light were nted by means of which the tip of the microphone probe could be carefully inserted into the external auditory meatus and in the other ear defender there was a hole for instruc tions to the patient when necessary during the examination

The pressure frequency response curve of the condenser microphone was an almost straight line according to its calibration chart in the range of 100–10 000 Hz with less than ±1 dB error, also as controlled after comple tion of this study The influence of the pre amplifier response was negligible when meas urements using a preamplifier were performed in the range of over 20 Hz (Bruel & Kjær 1972) The dynamic range of the microphone and preamplifier used for the bandwidth of 3 16 Hz at 1 kHz was from 1 dB to 160 dB sound pressure level (Bruel & Kjær 1975*a*)

The microphone was coupled into the ex ternal auditory meatus with a probe of suitable size A soft standard ear tip (Madsen Elec tronics A/S) at the end of the microphone probe was chosen to conform to the contours of the cartilaginous portion of the individual

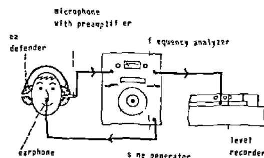


Fig 1 A block diagram of the equipment used in the present study See text

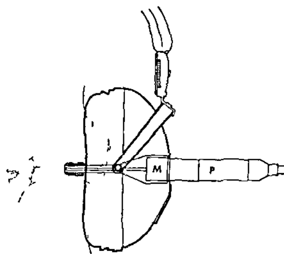


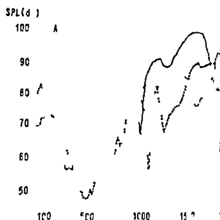
Fig 2 A sketch of the ear defender with a microphone (*M*) and a preamplifier (*P*)

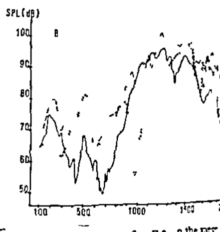
external auditory canal and was slightly compressible, ensuring a comfortable seal However, obtaining a truly air tight seal comparable to tympanometry was not necessary

The amplified output of the microphone was fed through a 3 16 Hz bandpass filter of the frequency analyser in order to suppress back und noise and the sound pressure level the filter section was recorded by the el recorder (Bruel & Kjær type 2307) By using various combinations of writing and paper speeds writing speed values of 1 250 mm/s and paper speed values of 10 mm/s were chosen permitting a sufficiently clear record of the essential features of the response On the other hand the filter function concerned with narrow band analysis results in a small amplitude error and time delay in writing out peaks (≈1 dB) (Bruel & Kjær 1975*b*) By means of the event marker facility of the level recorder operated manually by remote control the swallowing moments could be recorded

PRELIMINARY EXPERIMENTS

In order to evaluate the mechanics of sound transmission through the Eustachian tube during swallowing and to interpret the acoustic phenomena related to tubal function as meas





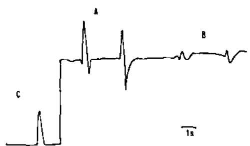
continuous line represent the same subject

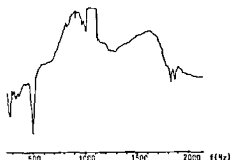
ured from the ear canal, the following ments were carried out

1 *Transfer function between nostril and ear canal in the range of 100–200*

A sinusoidal test sound was delivered nostril through the nasal olive and t quency response curve was record range of 100–2 000 Hz from both on 13 subjects and also from the other in 11 subjects by Heterodyne Analyzer & Kjær type 2010) without swall total normal adult group being 15 The subjects were sitting still in a without moving their heads or open mouths If the nasal olive was in both the mouth was slightly open for test

The frequency response curves





The tubal opening response as expressed with the ne of 400 Hz (A) 500 Hz (B) and without the test) and the frequency response curve in the range 000 Hz as measured from the ear canal of the same

rm in both groups but the levels of the -line were different depending upon her the test sound was picked up from the ralateral nostril or from the external tory meatus Fig 3 shows the frequency onse curve between 100 and 2000 Hz out swallowing (A) as recorded from the anal and (B) from the other nostril In the way a woman with a constantly patent tachian tube on one side was studied and response curves were basically alike in ear canals and the nostril There were resonance and antiresonance peaks in all ie recorded curves and they varied a great even in the same subject in successive surements The form of the transfer func was influenced primarily by the nasal oral cavities the nasopharynx and the rynx not so much by the Eustachian tube middle ear cavity or the external auditory al as shown by Eguchi (1975) too

Fig 4 shows how the test sound of 500 Hz (B) will be attenuated while the one of 400 Hz (A) describes very well the time of the tubal opening in successive swallowings The tubal opening response without the test tone (C) describes a component of 400 Hz from the swallowing sound, that was able to pass through the filter On the frequency response curve of the same person a proportionate antiresonance peak can be seen exactly at the point of 500 Hz

2 *Spectrum of swallowing sound*

The frequency analysis of the swallowing sound itself was measured from the external auditory meatus in 14 normal adults, 7 men and 7 women All had a negative history of recent airway disease and normal tympanic membranes Each person was asked to swallow either a sip of water or saliva several times while sitting in a relaxed position quietly and with the mouth closed The sound of swallowing was picked up by the microphone inserted into the circumaural defender and a third octave spectrum was analysed by means of Real Time 1/3 Octave Analyzer (Bruel & Kjær type 3347) from both ears of each subject This analyser performs a continuous real time analysis The integration time of the analyser was set so that the complete event was averaged and captured on the display

The principal shapes of the curves in all the swallowings were alike as seen on the display screen Of these recordings a random sample of 35 spectra was chosen and recorded with the level recorder The average was produced from the bargraphs and Fig 5 shows this mean curve According to this experiment the swallowing gave rise to broad spectrum noise The variations in the spectra of different swallowings of the same subject could be due to the volume changes of head cavities during the act of swallowing The variability was greatest in the range of 100–2000 Hz while above 7 kHz there were no frequencies on which the sound pressure exceeded the level of 30 dB

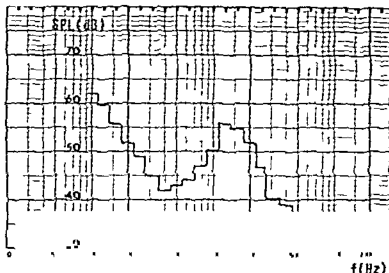


Fig 5 An average third-octave swallowing sound recorded by 1/3 Octave Analyzer from the [illegible] canal

The frequency response curves measured from the external ear canal and nostril were similar in form to the recordings of swallowing sound in the range 100–2 000 Hz Thus, the swallowing sound is also influenced by the resonance and antiresonance effects of nasal and oral cavities, hypopharynx and pharynx Based upon these data the frequencies most [illegible]e for the Eustachian tube opening meas[illegible]s would be on the high frequencies, [illegible]us from 5–6 kHz, where the intensity of swallowing sound is weakest

To clarify the usefulness of the high frequency test tone in connection with the mere swallowing sound the latter with tones 0 5 and 8 kHz were recorded on the sa[illegible] by using the Real-Time 1/3 Octave [illegible] Fig 6 shows that the test tone of 0 5 k[illegible] not be seen while the test tone [illegible] appeared separately from the cur[illegible] swallowing sound Broadband noise [illegible] used as the test sound The reson[illegible] antiresonance characteristics of head [illegible] alter the frequency spectrum of the [illegible] that it is in form similar to the cur[illegible] swallowing sound alone and together [illegible] broadband noise (Virtanen 1977) I[illegible] obtain supporting evidence that th[illegible] the frequency curve depends [illegible] resonance effects of the head the f[illegible]

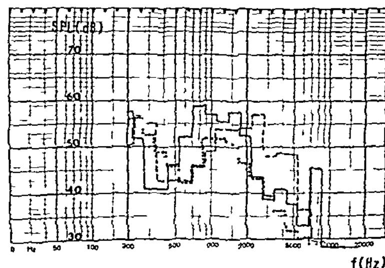
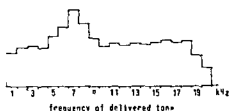


Fig 6 Sound spectrum as r[illegible] Real Time 1/3 Octave Analyz[illegible] external ear canal of the same [illegible] dotted curve results from the [illegible] sound only the interrupted [illegible] from the swallowing sound [illegible] tone of 500 Hz and the solid [illegible] from the swallowing sound [illegible] tone of 8 kHz



Tubal opening responses in 42 otologically nor rsons in frequencies 1–20 kHz

ysis of noise was recorded on a subject was able to keep the tube open volun- y and on a subject who had a patulous All the curves registered were alike in as compared with the situation of the ed tube, although there would be a direct open communication for the noise through tube between the nasopharynx and the dle ear (Virtanen, 1977)

ubal opening response in frequencies -20 kHz

ed on the above-mentioned result using the n frequencies for the sound conduction hod, measurements of the tubal function e made in the range 1–20 kHz on 42 normal ons with an age range between 6 and 55 s (mean age 32 years) The intratympanic pressure of each ear was determined with panometry resulting in normal V shaped panograms in all cases Since both Eusta in tubes were tested in each individual total number tested was 84 After deliver the test tone to the nose the subjects were t asked to swallow a sip of water and then va This was done at least once at all fre- ncies between kHz in the range 1–20 kHz or analysis of the results a positive opening ponse was registered if there was an in ase of 5 dB or more in the sound pressure el at the moment of swallowing It is prob- e that by using this criterion some smaller nings are overlooked, but pen excursions easily distinguished from the background activity recorded from the external auditory canal

Fig 7 shows the results of this material and it can be seen that the tubal opening was recorded best when using the frequencies 6 7 and 8 kHz

Eleven Eustachian tubes of 8 individuals (13%) showed no distinct opening response measured at 6, 7 and 8 kHz In five of these 11 Eustachian tubes there was a level increase of 2 to 4 dB and the opening response was easily distinguished from the flat base line of the background In the remaining six Eusta chian tubes (7%) it was not possible to record any kind of opening response in frequencies of 6, 7 and 8 kHz, but two out of these six had a positive opening response in higher fre quencies Thus only four (4 8%) had no meas- urable opening response in the range 6–20 kHz during swallowing at the moment of examina- tion

4 Test–retest reliability

Thirty six subjects (72 normal functioning Eustachian tubes) in the normal group were

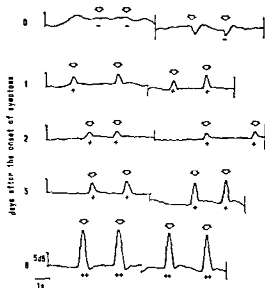


Fig 8 Opening of the Eustachian tube from the same ear during the course of nasopharyngitis Swallowing (of water and saliva alternately) is denoted by an arrow and subjective hearing by a plus sign The base line is 30 dB SPL at 7000 Hz

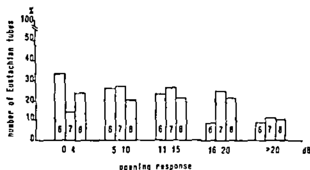


Fig 9 Opening responses of 106 normally functioning Eustachian tubes (on 60 subjects) classified according to the differing increases in level in frequencies of 6 7 and 8 kHz

studied in detail to test the reproducibility of the sonotubometric configuration The time between two examinations varied from 3 days to 6 months and the test tone was 7 kHz The difference of 0–5 dB in sound pressure level for tubal opening response between the two examinations was seen in 79% and the difference seen most frequently was 0–1 dB The difference was greater than or equal to 11 dB between two examinations in 2 8% only These results can be interpreted to show that within subjects the test–retest consistency is quite od

Sensitivity of test

order to verify the accuracy of this principle, experiments were performed during the course of nasopharyngitis Twelve persons with upper respiratory infection without earache, their ages ranging from 8 to 52 years, showed subjectively and objectively distinct signs of catarrhal condition in the middle ear the tympanic membrane was injected, retracted, or there was fluid in the middle ear Six of these patients had no hearing loss in their audiogram, an 11 year old boy had the largest conductive hearing loss, i e 10–20–10 dB in the speech area Three subjects had normal tympanograms 4 had a flat curve and 5 had V-shaped tympanograms the peak extending to 80 mmH$_2$O of negative pressure None of these subjects (12) could open their Eustachian tubes tested by the tone of 7 kHz during swallowing Fig 8 shows the opening of the Eustachian tube during the course of nasopharyngitis in one of the sub... by little, following the resolution of ... tion, the opening of the Eustachian t... turned to normal An observation ... made that no opening response of ... could be recorded if an otherwise ... ject reported a respiratory infection ... ing day It is thus possible that ... subclinical stage of an upper respira... tion the tube does not function in ... way

Next the tubal opening of the sub... had the negative intratympanic press... recorded This material consisted of ... and 24 Eustachian tubes, the age ... years, 9 males and 12 females In ... with the intratympanic pressure from ... mmH$_2$O no opening of the tubes ... recorded These observations are ... ment with those of Holmquist (197...) ... results indicated that middle ear ... levels outside a normal range of ±25 ... show poor Eustachian tube function

These experiments performed d... upper respiratory tract infection testi... sensitivity of this method and ... importance of investigating only patie... out catarrhal symptoms in order to ... true tubal opening results and to avo... negatives The same conclusion has reached previously by Flisberg (1963*a*, *b*), Andreasson & Ivarsson (197... Williams (1975)

Sonotubometric Results using 6–8 k... Tones in Normal Ears

This material consisted of 106 ears of ... jects The subjects ranged in age from ... years, with a mean age of 32 years (2... and 38 females)

After the patient had received ... instructions the test tones of 6 7 and ... were in turn applied to the nostril f... seconds and the patients swallowed a ... water from the mouth when the ... switched on a red light so that the s... ing moment was recorded on paper ...

marker of level recorder After each
ow of water the patient swallowed saliva
when instructed by the examiner This
gement was repeated three times at each
frequencies, 6, 7 and 8 kHz The subject
experienced a characteristic increase in
udness of the test tone during tubal open
After three water and three saliva swal-
gs the microphone probe was changed to
pposite external auditory canal and, by
ently using the same nostril, the proce-
was repeated The maximum amplitude
f these six swallowings was selected for
sis of results from each side Level
ges larger than or equal to 5 dB over the
line were accepted, because the smaller
onses could not always be distinguished
certainty from the fluctuating base line

e tubal opening recordings measured in
requencies of 6, 7 and 8 kHz (from the
normal Eustachian tubes on 60 indi
als) were subdivided into five groups ac-
ing to the increase in level (Fig 9) No
onse (≥5 dB) could be registered in 35
) for 6 kHz test tone in 14 (13%) for 7
test tone and in 25 (24%) examined ears
kHz test tone, and there was no demon
ble response (≥5 dB) in 11 (10%) ears for
frequencies of 6 7 and 8 kHz test tone
n (6 7%) out of these 11 revealed tubal
ing for the steady sound of higher fre
cies only In the remaining 4 Eustachian
s (3 8%) no opening response could be
rded (≥5 dB) at all during swallowing
e peak height of 1–4 dB was accepted
ubal opening provided it was easily distin
hed from the base line signal no opening
onse occurred in only 5% of normal ears
ng swallowing tested with 6, 7 and 8 kHz

COMMENT

n if earlier reports on the sound conduc
method have presented it as valid as used
in routine testing (Satoh et al , 1970)
y problems including a larger number of
tive responses (11–47%) have barred its
acceptance The other serious disadvantages of the sound conduction technique described earlier were its susceptibility to the different background noises, the arbitrary choosing of test frequencies and the fluctuation of the base line signal Many pitfalls existed, like the leakage of the sound through the tubes, the badly fitting ear plug lacking ability to achieve an airtight sealing in the external auditory meatus and the touching of the microphone tube The testing demanded a sound proofed booth and the microphone had to be shielded against noise

By holding the sound source near to the nostril, mounting the microphone into circum aural ear defender and by feeding the output of the microphone through a filter with an ex tremely narrow bandwidth (3 16 Hz) many of these pitfalls could be removed There was no need for a sound proofed booth, even the instructions could be given to the patient dur ing the examination when necessary, because the microphone system so constructed at tenuated the background noise by more than 30 dB and because the level of high frequency components in the normal speech spectrum is low

The recording of the transfer function of the passage between the nasal tip and the external auditory meatus revealed that some fre quencies were attenuated while the others were amplified This unequal attenuation of various frequencies could depend on specific physiological changes in volume and shape of the nasal cavity the oral cavity the naso-pharynx and the pharynx The recording of the spectrum of swallowing sound speaks in support of the existence of this resonance and antiresonance phenomenon This explains (Fig 4) why in earlier investigations there were many negative or sometimes debatable responses in otherwise otologically normal individuals

It became obvious from the present experi ments that the useful frequency range for the measurements by the sound conduction method was upwards from 5-6 kHz This was

presented in Fig 6, where the delivered tone of 500 Hz unsatisfactorily detected the tubal opening response, while the test tone of 8 kHz was easily distinguishable from the base-line One may reasonably expect that various kinds of resonance effects of head cavities also influence the higher frequencies (>5–6 kHz), but the wider dynamic range here makes it possible to record the acoustic phenomenon It is obvious that one simple frequency may not always give a reliable picture of the tubal opening, if the result proves negative By using the frequencies 6, 7 and 8 kHz (Fig 7) positive results (≥5 dB) showing a tubal passage were obtained in about 90% of normal ears If the peak height of under 5 dB is accepted for tubal opening, provided it is easily distinguished from the base line signal, the opening response occurred in about 95% of normal ears during swallowing In the normal group there can also be persons whose middle ear aeration is not achieved by swallowing but for instance by moving the mandible, by yawning, by blowing nose and by the Valsalva manoeuvre In Elner et al (1971) had cases (6 9%) in material of 102 ears which seemed to use technique other than swallowing for equilibrating

Perlman (1939) showed by means of sound conduction method that the tube does not open regularly to the same extent during each act of swallowing His recordings are in accord with the present study, but as a rule the tubal opening responses seemed in this material to be rather similar in the same patient during the separate swallowings, although each subject had a swallowing sound pattern of his own It may be that some persons can swallow better with a sip of water than with saliva but the pen excursions were of similar magnitude for both water and saliva swallowing Similar conclusions have also been made by Cole & Cole (1974) and by Williams (1975)

ZUSAMMENFASSUNG

Die Methode der Tondurchleitung zum Messen der Öffnung der Eustachischen Röhre wurde untersucht und das

schutzer eingebaut und der Output des M... einen Filter mit einer sehr engen Bandbre... geleitet wird konnten viele Mangel der T... technik die fruher beschrieben worden ... werden Tonaufnahmen der Übertragung... schen der Nasenspitze und dem äußeren Ge... Aufzeichnungen des Spektrums des S... gaben einen nutzlichen Frequenzbereich f... sungen von mehr als 5–6 kHz Bei einer ... der drei Frequenzen 6 7 und 8 kHz zeigte ... eine Tubenoffnung bei 90 oder 95% der norma... je nachdem ob als minimale Amplitude ... sprechend 1–4 dB angenommen wurde

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LONG-TERM OBSERVATION OF EARS WITH REDUCED MIDDLE EAR PRESSURE

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Abstract 335 ears from 210 children in which *tympanometry* initially indicated middle ear pressure ≤ -100 *mm* H_2O or a flat tympanogram have been reinvestigated 3 *years later* When the initial investigation was performed all children were 7 *years old The present* study reveals that 25% of these ears still have middle ear *pressure* ≤ -100 mm H_2O and 16% middle ear pressure ≤ -150 mm H_2O In normal material of the same age group 9% have *middle ear pressure* ≤ -100 mm H_2O and 4% *middle* ear pressure ≤ -150 mm H_2O The study also *reveals effusion* in 10% of ears with normal screening audiograms In ears with *normal* screening audiograms and middle ear pressure ≥ -145 mm H_2O *effusion* was found in 5% and in ears with abnormal screening audiograms and middle ear pressure ≤ -150 *mm* H_2O *effusion* found in 81% As tympanometric criterion *for oto*- referral middle ear pressure ≤ -150 *mm* H_2O *or a* tympanogram is suggested The inclusion of *tympano*metry in routine screening for middle ear pathology in children is recommended

Diagnosis and treatment of serous/mucoid otitis media is a routine problem for all otologists Many authors believe that serous otitis media predisposes the ear to adhesive processes chronic otitis media and cholesteatoma To prevent development of such chronic processes, early identification of this disease is certainly important Previous studies have demonstrated Eustachian tube dysfunction and subnormal middle ear pressure in all ears examined during serous/mucoid otitis media and in approximately 70% of the ears examined after this disease (see for example Renvall, 1975) However the long term importance of reduced middle ear pressure is not yet clear

The aim of this paper is to report a 3 year follow up of ears in which subnor[illegible] ear pressure was demonstrated d[illegible] ous/mucoid otitis media This obs[illegible] led to the development of middle ear [illegible] criteria for use in evaluating ser[illegible] otitis media

MATERIAL AND PROCED[illegible]

The material of this investigation is compr[illegible] in 210 children When the initial inve[illegible] performed all children were 7 years old and [illegible] evaluation they were between 10 and 11 years [illegible] the ear with a middle ear pressure of $\leq$ 100 [illegible] flat tympanogram at the initial invest[illegible] in this study Screening audiograms were [illegible] ears and were considered pathological if [illegible] thresholds exceeded 20 dB at two or more [illegible] The middle ear pressure as well as the [illegible] was measured with a Madsen impedance [illegible] (probe frequency 220 Hz) The pressure in the [illegible] canal was varied between −400 and +200 [illegible] ing the tympanogram recording The [illegible] threshold was determined by stimulating the [illegible]

[illegible]

stapedius reflex threshold of >95 dB H L was [illegible] abnormal Otologic examination of the ear was [illegible] by an experienced otologist

RESULTS

Otologic evaluation of these 335 ears [illegible] strated pathology (retracted or sti[illegible] effusion) in 25% of the ears ex[illegible] diometry and tympanometry show[illegible] mal findings in 16% while the stape[illegible] threshold was pathological in 61% of [illegible] See Table I

I *Findings in 335 ears in which inometry 3 years earlier demonstrated e ear pressure ≤−100 mm H_2O or a flat inogram*

	Normal	Abnormal
ic ination	(75%) 250 ears	(25%) 85 ears
gram ·ning)	(84%) 282 ears	(16%) 53 ears
inometry	(84%) 282 ears	(16%) 53 ears
ius reflex	(39%) 129 ears	(61%) 206 ears

ion related to audiogram and middle ear ure

sion were observed in 10% of 282 ears normal audiograms and 51% of 53 ears abnormal audiograms Tables II and III our basic observation of the relationship ffusions to otologic and audiologic find In those ears (256) with both normal mid-ar pressure (≥−145 mm H_2O) and normal ograms effusion was observed in 5% of cases while in those ears (27) in which a ologic middle ear pressure (≤−150 mm) and abnormal audiogram were identified, sions were observed in 81% of the cases isions were found in 54% of ears (26)

le II *Middle ear pressure and otologic ings in 282 ears with normal screening au-rams (a) and in 53 ears with abnormal ening audiograms (b)*

ion revealed by otoscopy in 27 ears of those with al audiograms (10%) and in 27 ears of those with rmal audiograms (51%)

H_2O	No of ears	Effusion (otologically)
lormal screening audiograms		
45	(91%) 256	(5%) 13
50	(9%) 26	(54%) 14
	282	27
lbnormal screening audiograms		
45	(49%) 26	(19%) 5
50	(51%) 27	(81%) 22
	53	27

Table III *Tympanometric and otologic findings in 335 ears in which tympanometry 3 years earlier indicated middle ear pressure ≤−100 mm H_2O or a flat tympanogram*

mm H_2O	No of ears	Effusion
≥−145	282	6%
≤−150 or flat tympanogram	53	68%

demonstrating pathologic middle ear pressure (≤−150 mm H_2O) and normal audiograms and 19% of ears (26) with abnormal audiograms and normal middle ear pressure

Effusion related to middle ear pressure

Tympanometry revealed abnormal middle ear pressure (≤−150 mm H_2O) in 16% in the present investigation (Table I) Six per cent of these ears with normal middle ear pressure (282) and 68% of ears with abnormal middle ear pressure (53) demonstrated effusions (Table III)

Analysis of ears with subnormal middle ear pressure and of ears with effusion demonstrated at myringotomy

Initial selection of cases for following study was based upon a criterion of a middle ear pressure ≤−100 mm H_2O As is shown in Table IV, 25% of the ears still demonstrated a middle ear pressure of this value or less Judged by our new criterion, 16% were patho

Table IV *Results from follow-up of 335 ears in which tympanometry 3 years earlier demonstrated middle ear pressure ≤−100 or a flat tympanogram compared with the findings in a nonselected group of 10-year-olds (206 ears from Renvall et al , 1973)*

Middle ear pressure (mm H_2O)	Selected group (no of ears)	Nonselected group (no of ears)
≤−100	(25%) 84	(9%) 19
≤−150	(16%) 53	(4%) 7

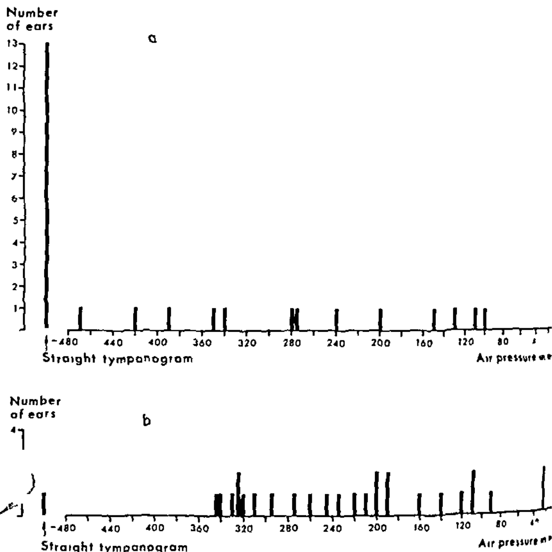


Fig 1 (A) Tympanometrically recorded middle ear pressure in 27 ears 1–2 hours prior to myringotomy Effusion could be demonstrated in all ears (B) Tympanometrically recorded middle ear pressure in 28 ears 1 [illegible] myringotomy No effusion could be demonstr[illegible]

logical In 55 ears in which the otologist recommended myringotomy, tympanometric recording was performed immediately prior surgery As seen from Fig 1 a straight tympanogram is more common in ears with surgically verified effusion than in ears without effusion However, 10 ears with effusion demonstrated a recordable negative middle ear pressure of ≤ -150 mm H_2O and in 4 ears with effusion, the middle ear pressure was > -150 mm H_2O

In the present investigation one ear has developed cholesteatoma and 13 ears retracted tympanic membranes or myringo[illegible] pexis (Table V) even though trea[illegible] the ears in question was performed [illegible] tion with the initial investigation

DISCUSSION

The diagnosis and treatment of serou[illegible] otitis media has been the subject of [illegible] tion during the last decade (Brook[illegible] Djupesland, 1969 Ferrer, 197[illegible] Wagoner, 1974, Muller & Geier 197[illegible] al , 1975, Lewis et al , 1976) In 19[illegible]

V *Otologic findings and screening au*
try in 53 ears with middle ear pressure
0 mm H_2O or a flat tympanogram

	No of ears	Normal	Retracted ear drum or myringo-stapedio-pexi	Effusion	Cholesteatoma
d	26	2	10	(54%) 14	
mal	27	1	3	(81%) 22	1

on culminated in a conference entitled ent advances in middle ear effusion', in h early detection of this disease was con- ed essential (Paparella, 1975) Since pure audiometry alone is not an adequate od for diagnosis of serous/mucoid otitis a (Brooks, 1968, Renvall et al, 1973, er & Wagoner, 1974, Renvall et al, 1975), est has been directed toward the de- oment of new diagnostic techniques The duction of impedance measurement pro- res in a form appropriate for routine clini- pplication has added a valuable diagnostic whose full potential still remains to be olished

this investigation it was shown that re- d middle ear pressure can be found in the ence of a normal screening audiogram) It is appropriate to question whether e children, who would be classified as nal on the basis of screening audiometric , only should receive further otologic ex- nation In Berry's study (1975) 5 of 7 ears h effusion had a middle ear pressure of $\leq$150 mm H_2O (660 Hz probe tone, admittance meter) and 49 of 66 ears with sion had a middle ear pressure of ≤ -150 H_2O (220 Hz probe tone) Our study re- ls that in over half of the ears with normal iograms and reduced middle ear pressure, sion was found (see Table II a) The detec- of one additional child in 11 (9%) with ormal middle ear function (Table II a) ild seem a sufficient argument for inclusion of tympanometry as part of any routine battery of screening tests

Additionally, in recently published long term studies of ears with serous/mucoid otitis media, adhesions were shown in 11 2% (Gundersen & Tonning, 1976) and 3 3% (Tos & Poulsen, 1976) of the cases reported and the incidence of cholesteatomas varied between 5 6% (Gundersen & Tonning, 1976) and 1 1% (Tos & Poulsen, 1976) These data indicate that ears with subnormal middle ear pressure constitute a risk group for the development of chronic middle ear disease Negative middle ear pressure of a certain magnitude may itself be responsible for development of retractions in the tympanic membrane (Tos & Poulsen, 1976) and when there is a retraction the ear has the potential to develop adhesions as well as cholesteatomas Typically the symptoms are minimal before this state of chronic middle ear disease develops Identification of this ear, predisposed to serious pathology, is difficult One possible means for such identification is regular, routine evaluation of ears in which subnormal middle ear pressure has been demonstrated Obviously, screening for such subnormal middle ear pressures is appropriate

Three considerations must be raised in the use of tympanometry to screen for middle ear abnormality

(1) In a temporal bone study (Renvall et al, 1975) it was shown that small amounts of water in the middle ear can change the impedance without flattening the tympanogram It is thus obvious that if the aim is to recognize effusion in the middle ear we must consider ears with recordable middle ear pressure as well as ears with a flat tympanogram Thus in 14 ears with effusion (Fig 1) a dip was recorded on the tympanogram and the middle ear pressure could be measured According to our criterion, 10 of these 14 ears with effusion showed abnormal middle ear pressure

(2) The criterion employed in the classification of an ear as normal or abnormal is critical We suggest that a criterion may be formulated

on the extent to which middle ear effusion is predicted by a given tympanographic finding Berry et al , 1975, Brooks, 1969, Grimaldi, 1976 have discussed the relevance of tympanogram shape Using as criterion a pressure of ≤ -150 mm H_2O we identified 23 of 27 ears with effusion (Fig 1) We suggest that such a criterion is easy to apply and may be more appropriate than sophisticated analysis of tympanogram shape, particularly in the use of this measure in screening tests

(3) Finally we note that reduced middle ear pressure can be a transient episode and require no treatment To avoid referring inappropriate patients to the otologist, repeated determination of middle ear pressure is suggested (Brooks, 1975, Liden & Renvall, 1977)

As is shown earlier by Renvall et al (1975) the reflex threshold is always higher than 95 dB H L or not elicitable in ears with middle ear pressure ≤ -100 mm H_2O In this study the stapedius reflex threshold was pathological in % *of the ears Therefore, the use of the* edius reflex test according to our criterion identification of ears with effusion is con idered too sensitive a test If, however, failure to elicit the reflex at 110 dB H L on two consecutive occasions is considered to be an indication for otologic investigation, this test must be discussed Brooks (1975) found that in an unselected material, around 15% belonged to this category However, the relation between middle ear pressure and pathologic stapedius reflex test must be further evaluated before we can decide whether the stapedius reflex test may add significant dimension to tympanometry (or indeed replace it) in our *efforts to detect serous/mucoid otitis media*

In such contralateral testing as we and others have used, it is often difficult to evaluate the extent to which each ear contributes to an abnormal finding The use of ipsilateral testing procedures may alleviate this difficulty With some concern for possible artifacts we are persuing ipsilateral testing procedures in our present and future studies

As mentioned earlier in this study one ear has developed cholesteatoma and [illegible] tracted tympanic membranes or [illegible] stapediopexis The important [illegible] however, is that 25% of these [illegible] still had a middle ear pressure of [illegible] H_2O and that 16% had patholog[illegible] pressure according to our criteria [illegible] selected group of the same age 97[illegible] strated middle ear pressure ≤ -100 [illegible] and 4% had pathologic middle ear [illegible] according to our criteria (See Table [illegible]) this follow-up shows that reduced m[illegible] pressure is more common in ears [illegible] had subnormal middle ear pressure [illegible] nonselected group Therefore regular [illegible] tion of patients with subnormal m[illegible] pressure is recommended This very [illegible] procedure may be one way to avoid [illegible] ment of chronic middle ear disease [illegible] often results in life long dependence [illegible] medical care

ACKNOWLEDGEMENT

Professor Josef M Miller Ph D reviewed the [illegible] script and gave valuable help This study was [illegible] by the Swedish Medical Research Council [illegible] 17X 133 10

ZUSAMMENFASSUNG

Bei 335 untersuchten Ohren von 210 Kindern [illegible] Tympanogramm einen Druck im Mittelohr [illegible] mm H_2O oder ein flaches Tympanogramm [illegible] sind nach 3 Jahren wieder untersucht worden [illegible] ersten Untersuchung waren die Kinder 7 Jahre [illegible] ben Kinder wurden nach 3 Jahren wieder [illegible] chung unterzogen bei welcher es sich zeigte [illegible] untersuchten Ohren weiterhin einen Druck [illegible] von ≤ -100 mm H_2O und 16% einen Druck [illegible] mmH_2O hatten Eine Untersuchung von [illegible] rial zeigte folgende Werte 9% hatten [illegible] ≤ -100 mm H_2O und 4% ≤ -150 mm H_2O [illegible] Untersuchungs befund bei normalen S[illegible] gramm mit einem Mittelohrdruck von > [illegible] zeigte bei 5% der Untersuchten eine Sekr[illegible] Mittelohr Bei Ohren mit abnormalem S[illegible] gramm und einen Mittelohrdruck von ≤ -1[illegible] zeigte sich eine Sekretbildung bei 81% [illegible] Studie zeigt daß bei Ohren mit normalem S[illegible] Audiogramm eine Sekretbildung bei [illegible] suchten Ohren vorkommt Als tympanometr[illegible] rium zur Überweisung an einen Ohrenspe[illegible] die Werte ≤ -150 mm H_2O oder ein flaches [illegible]

-vorgeschlagen Die Anwendung der Tympanomet _outine Screening bei Mittelohr Pathologie ist emp- vert

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BACTERIOLOGY OF THE CHRONICALLY DISCHARGING MIDDLE EAR

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Abstract Suitable bacteriological techniques revealed anaerobic bacteria in 38 (33%) of 114 chronically discharging middle ears The genus *Bacteroides* was cultured from 25 ears Aerobic bacteriology showed the predominance of staphylococci facultative enteric Gram negative rods diphtheroid bacilli and *Pseudomonas* species Anaerobic bacterial cultures were always mixed with aerobic bacteria 12 ears were culture negative and 9 of the 108 Gram stained smears revealed no bacteria No significant difference in bacteriology was noted between ears with or without local antimicrobial treatment or between profusely draining or only moist ears The ears with postoperative recurrent infection or with clinical suspicion of cholesteatoma grew anaerobes significantly more often and were seldom sterile Because [illegible]c bacteria are frequently associated with chronic [illegible] media their characteristics with regard to suscepti[illegible] to antimicrobials and to air must be remembered in the choice of therapy

The improved anaerobic bacteriological techniques of recent years have led to a re evaluation of the bacteriological etiology and emphasized the role of the anaerobes in many bacterial infections (Gorbach & Bartlett 1974) The foul smell of chronic ear discharge and the high frequency of anaerobic bacteria in otogenic intracranial infections (Heineman & Braude, 1963) suggest that anaerobes are a common occurrence in chronic otitis media Yet earlier methods have revealed only a few anaerobes (Palva et al 1971) or none at all (Friedmann, 1957 Wright 1970 Decher & Daum 1973, Cooke & Raghuvaran 1974 Deka & Kacker 1975) We therefore decided to study the bacteriology of chronic otitis media by using appropriate aerobic and anaerobic bacteriological methods

MATERIAL AND METHO[illegible]

The study was carried out on 10[illegible] tive out patients with active ch[illegible] media in a total of 114 ears 47 [illegible] had been previously subjected to [illegible] gery No dry ears were includ[illegible] study Bacteriological specimens w[illegible] under operation microscopic co[illegible] the same time the appearance of [illegible] discharge, and the status of the [illegible] membrane were inspected Since the[illegible] tion, 24 of the ears have been s[illegible] radical surgery, and cholesteatoma [illegible] in 18 of them

Specimens of the middle ear d[illegible] taken with a silver ear probe bef[illegible] or cleaning of the ear The spec[illegible] inoculated and spread on enriched [illegible] and neomycin vancomycin chocol[illegible] agar plates The plates were pre red[illegible] GasPak anaerobic system taken ou[illegible] onds before and placed back imme[illegible] ter the inoculation Aerobic spec[illegible] taken similarly on chocolated [illegible] plates which were put in a candle ja[illegible] tion at 37°C was started immediat[illegible] jars were later transported to the [illegible] logical laboratories Each specime[illegible] Gram stained The anaerobic tech[illegible] and the identification of anaerob[illegible] followed the lines proposed in the W[illegible] Anaerobic Bacteriology Manual (S[illegible] 1975)

The clinical findings the resul[illegible]

I Bacteria isolated from 114 consecu- ronic ears

	No of isolations
ic	
des fragilis	6
des melaninogenicus	16
des clostridiiformis	1
des sp *	6
terium necrogenes	1
terium sp *	1
cterium infantis	1
rium lentum	2
rium sp *	4
ibacterium acnes	1
ibacterium sp *	4
ium sporogenes	1
ium sp *	1
ella alcalescens	1
lla parvula	3
lla sp *	1
ccus magnus	5
ccus sp *	8
reptococcus sp *	2
c	65
and facultative	
chia coli	9
lla aerogenes	2
acter sp *	8
sp *	1
cter sp *	2
mirabilis	11
morganii	2
rettgeri	1
monas aeruginosa	7
monas sp *	9
philus sp *	1
bacter sp *	1
eroid bacillus*	34
subtilis	2
a sp *	8
ococcus aureus	32
ococcus epidermidis	27
occus*	1
lytic streptococcus*	2
	160

es not identified

and of the staining were all recorded ately The χ^2 test was used for the sta- l analyses between bacteriological and al classifications

RESULTS

al of 225 bacterial strains were cultured e I) 160 aerobic and 65 anaerobic (29%) genus *Bacteroides* accounted for 45% (29/65) of the anaerobes, *Bacteroides melaninogenicus* being cultured 16 times The second in order of frequency was the genus *Peptococcus*, found in 13 cultures (20% of the anaerobes) The aerobic bacteriology showed the predominance of staphylococci (59), facultative enteric Gram negative rods (36),

stresses their importance in the infective process of a chronic ear

Anaerobic bacteria grew in 38 ears (33%), always mixed with aerobic bacteria Each ear contained 3 4 species on average, 1 7 anaerobic and 1 7 aerobic *Bacteroides* species were cultured from 25 ears, that is, from 22% of all the ears anf rom 66% of the ears with an aerobic bacteria 12 ears (11%) were culture-negative

Gram stained smears were obtained from 108 ears, in 98 (91%) of them bacteria were found (Table II) Seven of the 108 ears (6%) were devoid of bacteria both in the culture

Table II *Gram staining in 108 chronic ears*

Gram	No of identifications
Gram negative rod	64
Gram positive rod	42
Gram negative diplococcus	16
Gram positive coccus	55
Total	177

Table III *Bacteriological finding and local antimicrobial therapy*

Bacteriological finding	No of ears Drops	No drops	Total
Anaerobic			
Mixed with aerobic	18	20	38
Aerobic			
Mixed	10	15	25
Pure	25	14	39
No growth	9	3	12
Total	62	52	114

Table IV *Bacteriological finding and middle ear discharge*

Bacteriological finding	No of ears Purulent	Intermediate	Moist	Total
Anaerobic				
Mixed with aerobic	29	5	4	38
Aerobic				
Mixed	19	4	2	25
Pure	27	7	5	39
No growth	4	2	6	12
Total	79	18	17	114

Table VI *Bacteriological fin[illegible] perforation of the Tympanic membr[illegible]*

Bacteriological finding	No of ears [illegible] Attic/postero-superior tensa	Other [illegible]
Anaerobic		
Mixed with aerobic	11	20
Aerobic		
Mixed	4	17
Pure	4	3[illegible]
No growth	0	11
Total	19	80

and in the Gram stain Parallel to the culture results, most of the stainable bacteria were seen in abundant numbers By morphology, a total of 177 bacteria were traced in the smears In 31 ears (29%) the culture and staining results agreed, in 54 ears (50%) the smears showed bacterial species not found in the culture and in 47 ears (44%) the culture revealed bacteria not seen in the smears Altogether the stain revealed 69 bacteria not dis[illegible]ed by the culture 15 of the stainable [G]ram negative diplococci were unverified in culture, and in other Gram groups roughly one third of the bacteria remained culturally unverified Further, all the 12 cultured diplococci (4 *Veillonellae* and 8 *Neisseriae*) failed to stain, in other Gram groups the share was again roughly one-third Of the 39 ears with pure culture, 20 showed additional b[illegible] the smears, making monoinfection [illegible] rare (19/114=17%) in chronic otitis m[illegible]

62 of the ears had been treated [illegible] drops within the previous 12 hours (T[illegible] This did not correlate significantly [illegible] bacteriological culture findings alth[illegible] was a trend towards more abundant b[illegible]logy in the untreated group As re[illegible] appearance of the middle ear disch[illegible] bacteriology was essentially the sa[illegible] groups, but sterile cultures conce[illegible] nificantly ($P<0\ 001$) in cleaner ears (T[illegible] In the group with postoperative re[illegible]fection there were more anaerobic [illegible] sterile cultures than in the unoper[illegible] (Table V) Both these findings w[illegible] significant ($P<0\ 05$) Perforation of [illegible]

Table V *Bacteriological finding and previous ear surgery*

Bacteriological finding	No of ears Operated	Un operated	Total
Anaerobic			
Mixed with aerobic	20	18	38
Aerobic			
Mixed	11	14	25
Pure	14	25	39
No growth	2	10	12
Total	47	67	114

Table VII *Bacteriological find[illegible] [chol]esteatoma (operatively verified)*

Bacteriological finding	No of ears Cholesteatoma	No chol[illegible]
Anaerobic		
Mixed with aerobic	9	2
Aerobic		
Mixed	2	0
Pure	6	4
No growth	1	0
Total	18	6

membrane was attic and/or postero or in 19 ears These showed significantly anaerobic bacteria ($P<0.01$) and were often sterile ($P<0.05$) than those with perforations (Table VI) 24 of the ears been operated since the bacteriological nation (Table VII) Anaerobic bacteria cultured from nine of the 18 cholesteato is ears against two of the six ears with holesteatoma Although the groups were mall to show statistical significance an ic bacteria seemed to concentrate in holesteatomatous ears

DISCUSSION

present study confirms our recent finding pu et al 1977) that anaerobic bacteria an important component of the bacterial in chronic otitis media Our finding of robic bacteria in 33% of the ears contrasts the earlier findings of up to 1 1% (Palva 1971) a common development in other s of infections as well suggesting the in uacy of the earlier methods of investiga (Gorbach & Bartlett 1974) Even the Pak system with pre reduced anaerobic ire media and bedside inoculations used may lose the most fastid ous anaerobes sche 1969) and probably the real number naerobic bacteria in chronic otitis media is greater than we discovered This is also ested by our finding of stainable bacteria traced by the present culture method in a of the ears studied However the inci e of anaerobic bacteria was exactly the e as in the smaller material analysed by group (Jokipii et al 1977) a fact suggest he reliability of the method we used

lthough the pathogenicity of most of the ortunistic anaerobes presented here is rec ized (Zabransky 1970) the role of an obic bacteria in chronic otitis media cannot be established However in infections sed by the normal flora of the skin and ous membranes anaerobes are thought e important pathogens (Gorbach & Bartlett 1974) Chronic otitis media may be classified among these infections

The anaerobes always grew in mixed cultures Facultative bacteria are believed to act synergistically in mixed infections they remove oxygen produce substances that lower the redox potential of the tissues or provide nutrients that are necessary for the proliferation of the anaerobic pathogens This kind of bacterial synergy is probably common in anaerobic infections particularly in superficial infections such as otitis media

The bacteriology did not differ significantly between the groups with or without local antimicrobial therapy Because the groups were not random samples this only means that the ear drops used did not sterilize the ear Furthermore (anaerobic) bacteria were as frequently seen whether the ear drained profusely or was only moist

The ears with postoperative recurrent infection or with clinical suspicion of cholesteatoma (attic or posterosuperior perforation of the drum) showed anaerobic bacteria significantly more often and were seldom sterile in the present material Thus anaerobic bacteria were especially associated with the ears having the most troublesome disease problems Although it can be speculated that the anaerobes proliferate more readily in the ears with the most troublesome pathology the relationship does not imply an etiologic role of the anaerobic bacteria in these processes

The aim of conservative treatment in active chronic otitis media is to eradicate infection and to make circumstances for future operation as favourable as possible This is achieved by mechanical cleaning of the middle ear and by local antimicrobial treatment Systemic antimicrobials are needed only during some acute stages of chronic otitis media and during per and postoperative treatment of the ear When antimicrobial therapy is being considered the characteristic susceptibility of the anaerobes to various antimicrobial agents must be remembered When the treatment is local this feature of the anaerobes is especial

ly noteworthy. For instance, all the anaerobes are resistant to aminoglycosides, of which neomycin and gentamicin are commonly used ototopically. The different effects of these as well as many other antibiotics on different kinds on bacteria, and their suspected ototoxicity (Mittelman, 1972) offset the questionable benefits achieved by their ototopical use (Lancet, 1976). Consequently, in the treatment of chronic otitis media, the importance of careful and repeated mechanical cleaning of the discharging middle ear is further emphasized.

ZUSAMMENFASSUNG

Durch geeignete bakteriologische Verfahren wurden anaerobe Bakterien bei 38 (33%) von insgesamt 114 chronisch eiternden Mittelohren festgestellt. Die Gattung *Bacteroides* wurde von 25 Ohren kultiviert Unter den aeroben Bakterien waren Staphylokokken, fakultative enterale gramnegative Stäbchen, Diphtheroide und Pseudomonaden vorherrschend Anaerobe Bakterien wuchsen immer als Mischflora zusammen mit aeroben Bakterien 12 Ohren waren kulturnegativ und bei Gram-Färbung wiesen 9 von 108 Proben keine Bakterien auf In der Bak- logie gab es keinen signifikanten Unterschied zwi- Ohren mit und ohne örtliche antimikrobische Be- ng Auch war kein Unterschied zwischen profus den und nur feuchten Ohren festzustellen In Oh- n mit postoperativ wiederkehrender Infektion oder mit klinisch suspektem Cholesteatoma wurden signifikant mehr anaerobe Bakterien beobachtet, und sie waren selten steril Weil anaerobe Bakterien haufig bei chronischer Mittelohrenentzündung angetroffen wurden, mussen ihre Antibiotika und Luftsensibilität bei der Wahl der Behandlung berucksichtigt werden

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COMPUTER ANALYSIS OF OPTOKINETIC NYSTAGMUS IN PATIENTS WITH SPONTANEOUS NYSTAGMUS OF PERIPHERAL VESTIBULAR ORIGIN

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r The bias of slow phase velocity (SPV) of opto-
iystagmus (OKN) caused by an acute labyrinthine
/as examined in 8 patients using different opto-
stimulus velocities In all patients a directional
lerance of OKN–SPV was found corresponding to
ieous nystagmus This was due to enhancement of
ius SPV to the side of the lesion and depression of
the opposite horizontal direction The preponder
OKN on the average increased with the intensity
taneous nystagmus and decreased along with re
These vestibularly induced differences in OKN–
nge up to 70% A differentiation is discussed be
OKN preponderances caused by labyrinthine
and brain stem lesions

tional preponderance of optokinetic nys-
is may be observed in patients with an
unilateral labyrinthine lesion In these
its the vestibular imbalance not only
's spontaneous nystagmus but also biases
inetic nystagmus (OKN) (Ohm, 1932,
& Mittermaier, 1939, Suzuki & Komat-
1962, Guttich & Okamoto, 1964, Coats,
The observations reported in the litera-
suggest an enhancement of OKN slow
velocity if spontaneous nystagmus beats
same direction as the OKN saccades, and
ression if it is in the reverse direction Ex-
for a few cases reported by Guttich &
noto (1964) there are, however, no quanti-
data about this interaction For the clini-
it is important to differentiate between di
onal preponderances of OKN due to an
iteral brain stem lesion and an exclusively
nthine defect A concomitant brain stem
n cannot be excluded if only depression of
I–SPV occurs The preponderance may be recognized as "benign" and of peripheral vestibular origin if OKN is both depressed and enhanced by the vestibular imbalance We therefore consider it useful to present briefly both computer evaluations of OKN slow phase velocity (SPV) influenced by labyrinthine lesions and a quantification of the directional preponderance with respect to the SPV of spontaneous nystagmus

METHODS

Patients

Six female and 2 male patients, 19 to 52 years old, volunteered for this clinical series of experiments All of them suffered from an *acute unilateral peripheral vestibular lesion* and initially complained of severe vertigo, gait disturbance, oscillopsia, and motion sickness Four of the lesions were on the right side and four on the left Each was interpreted to be either traumatic, vascular, or inflammatory in origin The diagnosis was based on a careful neurological and electronystagmographical examination including the demonstration of unilateral labyrinthine underexcitability by caloric testing (Fitzgerald & Hall-

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pike 1942) Two patients could be investigated repeatedly during the course of their recovery (be it by central compensation—Bechterew, 1883, Schaefer & Meyer, 1975—or by restitution of labyrinthine function) None of the patients gave indications of having additional brain stem, cerebellar, or cortical disturbances

Apparatus recordings of eye movements, and data analysis

The experiments were performed using a modified version of the Tonnies Electronystagmography System This consisted of a servocontrolled, motor driven rotary chair located in front of a large segment of a cylindrical screen the longitudinal axis of which was centred with respect to the subject s head The subjects sat in the upholstered chair fitted with a head support so that the screen with its 79 5 cm radius of curvature subtended a visual angle of about 100° vertical and 140° horizontal extent An optokinetic stimulator was used to ntinuously project a regular pattern of verti y oriented black and white stripes each 7 5° ide These moved horizontally at constant angular velocities of 30° 60°, 100°, and 120°/sec respectively to either the left or right The optokinetic stimulus field could be restricted in its horizontal dimensions to a visual angle of 30° by partial covering of the stimulator small field stimulation

Horizontal eye movements were recorded using silver–silver chloride surface electrodes (Beckmann) placed over the outer canthus of each eye After d c amplification the signals were stored on a 7 channel FM tape recorder (Bell and Howell VR 3200) for subsequent computer analysis

Calibrations of eye movements were taken from voluntary saccades of increasing visual angle (15°, 20°, 30°, and 40° to the left and right) which were separately least mean squares fitted for right and left calibrations by two cubic curves The signals of eye movements recorded on tape were analog to digital converted and stored on digital computer disks (sampling interval 10 msec) The re[illegible] analysed using a modified comp[illegible] adapted from MITNYS II (Allum et [illegible] on an IBM 1130 computer This prog[illegible] matically computed for every 10 ms[illegible] the slow phase velocity (SPV) of [illegible] nystagmus, which was averaged ov[illegible] intervals Where a saccade occurred [illegible] tude, duration and maximum avera[illegible] were also analysed Because of th[illegible] storage capacity of the computer [illegible] of optokinetic nystagmus could be [illegible] at one time Curves displayed on [illegible] plotter presented the original nyst[illegible] tern together with the actual sl[illegible] velocity as well as occurrence and [illegible] fast phases Computer printouts [illegible] tained the 4 8 sec slow phase vel[illegible] ages

For quantitative evaluation of OK[illegible] eventual directional preponderance t[illegible] interval with the lowest coefficient [illegible] tion (standard deviation divided by [illegible] SPV within the recorded period of [illegible] first determined Using these interva[illegible] difference in OKN slow phase velo[illegible] two horizontal directions was then [illegible] as a percentage of the faster OKN

Experimental procedure

After calibration of horizontal e[illegible] ments spontaneous vestibular nystag[illegible] recorded first with the eyes closed [illegible] intensity of spontaneous nystagm[illegible] dependent on vigilance subjects we[illegible] to perform mental arithmetic S[illegible] nystagmus was again recorded at th[illegible] each experiment

Directional preponderance of optokinetic nystagmus

(OKN) was then tested by asking [illegible] to follow the moving stripe pattern a[illegible] Two 20-sec periods of optokinetic n[illegible] were recorded for each constant velo[illegible] ulus speed (30° 60° 100° and 1[illegible] each horizontal stimulus direction T[illegible]

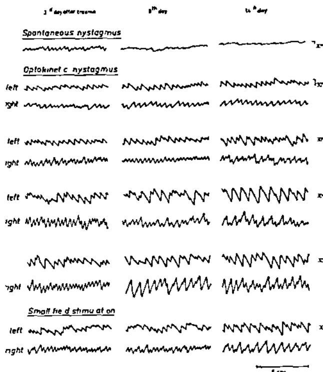


Fig 1 Original recordings of the horizontal component of spontaneous nystagmus and optokinetic nystagmus 3 9 and 14 days after a right traumatic labyrinthine lesion Directional preponderance of OKN toward the side of spontaneous nystagmus increases with increasing stimulus speed and progressively vanishes with compensation for the vestibular imbalance

arated by 30–60 sec relaxation periods ally OKN to the left and to the right was ed with the small field of stimulation at an ular velocity of 60°/sec

RESULTS

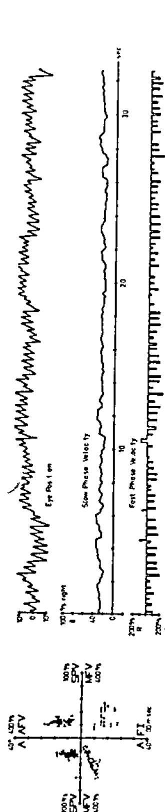
ontaneous vestibular nystagmus

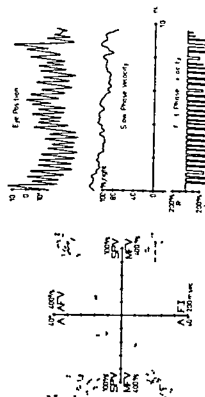
patients investigated exhibited spontaneous nystagmus This was invariably di ted towards the side opposite to the oned labyrinth The average slow phase ocity (SPV) of spontaneous nystagmus over registration period of 20 sec ranged from ° to 32 7°/sec The 2 patients who were eatedly studied during recovery showed a gressive decrease in SPV of spontaneous tagmus

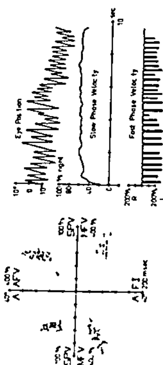
requently the SPV of spontaneous vestular nystagmus exhibited a considerable iance within and between several trials in subject This happened despite the atnpt to maintain vigilance by asking the subjects to perform mental arithmetic For the evaluation of the consequences of vestibular imbalance on optokinetic nystagmus the 11 recordings from 8 patients were arbitrarily divided into two groups of spontaneous nystagmus intensity The dividing criterion was whether the average slow phase velocity was above or below 12°/sec The average values of group 1 were 2 5°, 7 8°, 11 1°, 11 2°, 11 3°, 11 9°, 11 9°/sec Those of group 2 were 12 7°, 15 0°, 22 9°, 32 7°/sec

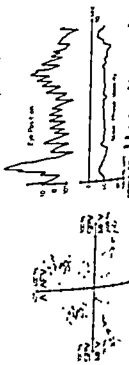
Optokinetic nystagmus

All patients tested consistently revealed a directional preponderance of OKN This was always toward the direction of spontaneous vestibular nystagmus and occasionally reached 65–75% in cases with a severe vestibular imbalance The original recordings in Fig 1 and the computer traces of slow phase velocity in Fig 2 may serve as an illustration Their evaluation together with the data

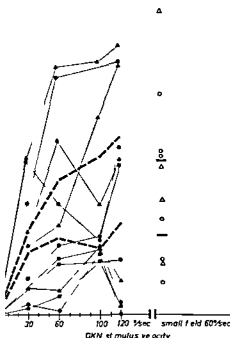








OKN 120°/sec stripes left



Percentual OKN preponderance (*ordinate*) in rela
) stimulus velocity (*abscissa*) Individual data of
ts with spontaneous nystagmus—SPV exceeding
: (*triangles*) and those with smaller spontaneous
dots) Dashed lines show the averages of the two
s at each stimulus velocity Corresponding data for
field stimulation on the right

:ted in Fig 3 allow of the following addi-
il statements

) For a given spontaneous nystagmus the
:tional preponderance of OKN slow phase
city tends to increase at higher stimulus
ds The preponderance may be absent or
k at lower stripe velocities of 30°–60°/sec
ıg a small field of 30° horizontal extension
optokinetic stimulation, the percentual dif
nce between the two directions becomes
ut as large at 60°/sec as with full field

.2 Example of a computer evaluation of nystagmus
ı plot contains the original eye movements a con
ous evaluation of slow phase velocity (*SPV*) and the
ities of fast phases Data depicted in cross co-
ıates on the left relate SPV to amplitude (*A*) and
age velocity (*AFV*) of fast phases as well as maximum
phase velocity (*MFV*) to amplitude (*A*) and interval
st phases (*FI*)

stimulation at 100° or 120°/sec, although the absolute difference is not as great This is illustrated by the averages presented in Fig 3 In four cases with moderate spontaneous nystagmus (slow phase velocity) the directional preponderance was so weak that it did not fall outside the range of normal variation (15%–22%)

(*b*) The percentual difference of OKN slow phase velocity to the right and to the left increases on average with the mean slow phase velocity of spontaneous nystagmus This is documented in Fig 3, by the difference between the two intensity groups For group 2 presenting the more vivid spontaneous nystagmus the difference reached a mean of 40% at 120°/sec stimulus speed whereas the cor responding values in group 1 hardly exceeded 20% There is however no close correlation between vestibular nystagmus intensity and the degree of OKN directional preponderance, a fact that hampers diagnostic evaluation in individual patients

OKN directional preponderance during compensation for a peripheral labyrinthine lesion

The difference in OKN–SPV generally decreases with decreasing SPV of spontaneous nystagmus during recovery from a peripheral labyrinthine lesion So far, only the percentual differences of right and left OKN were discussed in cases of spontaneous nystagmus For the clinician and the physiologist, however, it is of great interest to know whether this difference is due to a facilitation of OKN to the side opposite to the lesion, or an inhibition of OKN to the same side, or both If it were due to inhibition alone, it would be very difficult for the clinician to decide whether the OKN diminution toward the side of the lesion was due to an additional brain stem lesion or not From the absolute data it is difficult to answer, since, among the cases studied, none exhibited an OKN–SPV exceeding stimulus velocity, although initially they had abnormally high values of SPV The fact that in a

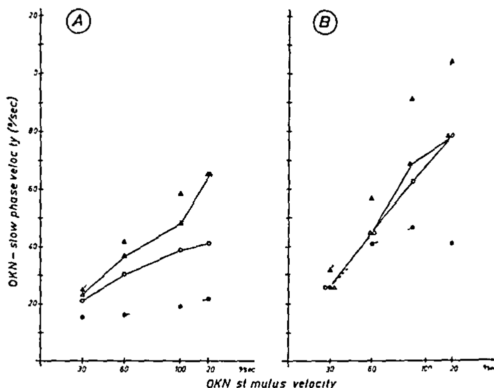


Fig 4 OKN slow phase velocity (ordinate) in relation to stimulus velocity for 2 individual subjects (A B) The initial recordings after labyrinthine lesion (▲ ●) show a significantly greater difference in SPV than those obtained 11 days later (△ ○) when spontaneous n[illegible] creased to one third Data indicate a [illegible]ment and depress[illegible]n of OKN

instances slow phase velocities of OKN reached 110°/sec at 120°/sec stimulation velocity when OKN was toward the direction of spontaneous nystagmus may indicate facilitation The exceptionally low slow phase velocities of OKN opposite in direction to spontaneous nystagmus (i e 40°/sec at stimulus velocities of 120°/sec) certainly prove inhibition A stronger argument for the occurrence of both facilitation and inhibition of OKN is given by the results of repeated measurements during the course of recovery from or central compensation for a peripheral labyrinthine lesion As can be seen from Figs 1 and 4 slow phase velocity of enhanced OKN decreases with the diminution of vestibular imbalance as judged by spontaneous nystagmus–SPV while SPV of the depressed OKN increases It may consequently be assumed that the basically normal performance of the optokinetic system was biased both ways by the vestibular imbalance

COMMENT

The results substantiate the old [illegible] that vestibular imbalance may [illegible] depress optokinetic nystagmus (O[illegible] Jung & Mittermaier 1939 Suzuki [illegible] suaki 1962 Guttich & Okamoto 19[illegible] 1968) The data agree with observa[illegible] mals (Koenig et al 1978) wh[illegible] variable gain of a transient vest[illegible] tion effect dependent upon optok[illegible] tern velocity The vestibular [illegible] (OKN preponderance) is found [illegible] with increasing optokinetic pat[illegible] the retina and with the strength of [illegible] imbalance both for the mutually e[illegible] the depressing combination of [illegible] interaction is thus not just purely [illegible] subtractive but a feed forward op[illegible] control of the vestibular com[illegible] plication) is involved before the [illegible] are combined It may be hypothes[illegible] gain control is performed by th[illegible]

ellar flocculus receiving visual input ion & Alley, 1974) It seems to involve itional individual component, since the tative correlation between spontaneous mus intensity and the consequent direc preponderance of OKN is rather vari etween individuals Some other mech must be responsible for the bidirectional sion of OKN-SPV following bilateral ithine lesions (Zee et al , 1976)

ctional preponderance of optokinetic mus in the direction of spontaneous mus is a frequent observation in pa- with acute peripheral vestibular lesions directional preponderance, however, not always exceed the range of pre- rances observed in normals Depending e stimulus condition the normal range between 15% and 22% (Dichgans et al ,

A combined lesion of the homolateral ie recticular formation may cause a simi ectional preponderance but this will only e to diminution of OKN to the side op- e the lesion and not to an enhancement to pposite side A comparison between the recording performed in the acute phase a second one performed 2 weeks later ates the vestibular origin of an OKN nderance by exhibiting a reduction of enhancement along with the compensa- for the vestibular imbalance The best ate of a possible OKN preponderance, in absolute and relative terms, is ob- d by optokinetic stimulation either at sec with an ample stimulus or with a small at 60°/sec (Dichgans et al , 1974) Un- ly fast SPV and high frequency of OKN rd the side of spontaneous nystagmus al suggest that the OKN preponderance is solely to vestibular imbalance and not to a stem lesion OKN in patients suffering a labyrinthine lesion is not purely visually n but must be correctly termed *vestib biased OKN*

this context one may recall that gaze nys us in the direction of spontaneous nystag- may be another consequence of periph eral vestibular lesion and not due to involve- ment of the brainstem

ZUSAMMENFASSUNG

Vestibulare Tonusdifferenzen nach akuten einseitigen Labyrinthlasionen führen zu einer richtungsbestimmten Beeinflussung der Geschwindigkeit langsamer Phasen des optokinetischen Nystagmus (OKN) Dies wurde bei acht Patienten zum Teil wiederholt quantitativ untersucht Alle zeigten ein Richtungsuberwiegen des OKN in Richtung des Spontannystagmus das auf Bahnung des ipsiversiven und Hemmung des kontraversiven OKN beruht Das Richtungsuberwiegen nahm im Mittel mit der Intensitat des Spontannystagmus zu und mit dessen Kompensation ab Es erreichte maximal 70% Eine Abgrenzung gegen Optokinetik Storungen bei Hirnstammlasionen ist moglich

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VERTIGO AS REFLECTED BY THE NYSTAGMOGRAM

A Clinical Analysis

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Efforts to evaluate findings in the nystagmo- ere made in a material of 338 vertiginous and atients A spontaneous nystagmus a positional ius as well as a difference in caloric reactivity are iry findings of little value for revealing peripheral s On the other hand central disturbances are fre revealed by inability to track a moving optic tar ilting in an irregular or a saccadic pattern by per of vestibular nystagmus in light by persistence nystagmus at eye-closure by an increase in spon nystagmus on eye-closure or by dysrhythmic nys- in caloric tests Cases of functional vertigo seem to om cases of vertigo from other sources by their of spontaneous nystagmus (when present) or by nce of caloric nystagmus on eye-closure

ee preceding papers a material of 338 inous and dizzy patients was analysed respect to the character of perceptions iksson et al , 1976*a*), to duration of per ins (Henriksson et al , 1976*b*) as well as nificance of functional symptoms (Afze t al 1977) In the present paper these s will be related to the nystagmographic gs

spite of excellent works on nystagmogra Aschan et al , 1956, Jongkees & Philips 1964, Megighian 1959) many positive igs are not entirely understood and their al interpretation is still doubtful The aim e present paper is therefore to deepen nderstanding of these findings by looking ossible correlations between various ob- e findings and also to correlate these igs to perceptions and symptoms Access ta from an analysis of 338 patients (re- d in our previous papers) with data added complete routine nystagmograms forms asis for our contribution

MATERIAL AND METHODS

338 patients consulting our "Dizzy clinic" were carefully investigated, with anamnesis, otological examination as well as nystagmography, as described in previous papers

The nystagmographic examination com prised the following procedures, previously comprehensively accounted for by one of us (Henriksson et al , 1967, Henriksson et al , 1972)

I *Tracking test*

In this test the patient was trying to follow with his eyes a target moving at constant angular velocity on a circular screen one metre in front of him Pathological findings were 1, Irregular tracking patterns 2, Saccadic tracking pattern

II *Spontaneous eye movements*

Test in darkness for spontaneous eye movements with open and closed eyes for 1, Spontaneous horizontal nystagmus 2, Undulating eye movements 3, Effect of eye-closure on spontaneous nystagmus

III *Caloric nystagmus responses*

(open eyes in darkness)

This test was undertaken for 1, Difference in caloric reactivity 2, Persistence of caloric nystagmus at eye closure 3, Dysrhythmia

A test for gaze- and positional nystagmus was also made

Information from the questionnaire and from the nystagmogram was fed into a com

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Fig 3 Undulating eye movements behind closed eyes

ents of the target In between these s the eyes moved slower than the tar could in some cases be nearly or com at a standstill This pattern of eye ent gave the recording a very charac appearance (Fig 1*E F*)

iency Thirteen patients had a slight e pattern and 7 had pronounced sac

lations Patients with gaze nystagmus ntly had saccades in the tracking tests mpared with 1% among the other pa 5% significance) Further the saccades lso significantly often combined with of inhibition of vestibular nystagmus (high ocular fixation index according to ez & Ledoux 1969) All 5 serious cases istic neurinomas 3 cases of multiple is) in this material were found among atients with pronounced saccades

pretation Gaze nystagmus and sac are positively correlated They may re milar but possibly not always identical iances of eye motor coordinating struc The clinical importance of pronounced es almost proving serious central dis must here be stressed it must be men however that also only moderate use of urates or other sedatives may cause this hic tracking pattern

II *Spontaneous Eye Movements*

ontaneous hori ontal nystagmus

rance Spontaneous nystagmus may be o detect when not complicated by simultaneous eye blinks The derived recording technique frequently helped by reflecting the fast component as a conspicuous spike

Frequency 72 patients had spontaneous nystagmus (open eyes in darkness) Nystagmus beats appearing only behind closed eyes were not included as they may well be looked upon as normal findings

Correlations Only one significant correlation between existence of spontaneous nystagmus and other signs or symptoms was found Patients with spontaneous nystagmus had significantly fewer undulating eye movements behind closed eyes than other patients 3% against 14% among other patients

Interpretation The lack of correlation to other signs and symptoms is interesting Nystagmus is then—although it represents an objective background for vertigo—of little value for reaching a diagnosis or determining whether a central or a peripheral source of vertigo may be expected The negative correlation to undulating eye movements is explained by the fact that nystagmus decreases in sleepiness (Collins & Guedry 1962) and by the fact that undulations are frequent in sleepy patients (Hamersma 1957)

2 *Undulating eye movements*

Appearance Undulating eye movements were frequently of large amplitudes and with a frequency of 0 2–0 5 periods/second (Fig 3)

Frequency Undulating eye movements behind closed eyes were observed in 38 patients gazing straight ahead and in another 29 pa

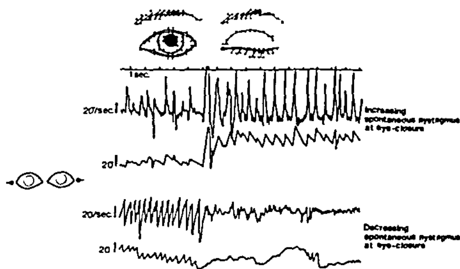


Fig. 4. Incr... creasing spon... tagmus ...

tients there were such movements only when gazing to the right or to the left.

Correlations. Apart from the above mentioned negative correlation to spontaneous nystagmus, patients in this group differed from other patients only with respect to certain expressions of social adaptation. They were thus more frequently divorced: 16% against ...% for other patients, their conjugal relations were less good: 8% against 2%, and they regarded their economy as poor, 21% compared with 11% among other patients.

Interpretation. The negative correlation between spontaneous nystagmus and undulating eye movements was discussed above. There is general agreement that these eye movements express sleepiness (Aschan & ... Aschan, 1967; Clement, 1970; E... 1957). Sometimes in patients with ... disturbances undulations are ... expressions of a pathological sleep... riksson).

The relation between presence of ... tions and a poor social adaptation ... difficult to interpret. Possibly ... cidental significance. However ... raises the question whether the ... ness denoted here by the presence of ... tions also indicates a certain social ... ment.

3. *Effect of eye closure on spon... nystagmus*

Appearance. Spontaneous nystagmus ... quently rather heavily modified by ... (Fig. 4).

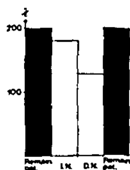


Fig. 5. Number of early functional symptoms in % in patients with increasing (I.N.) and decreasing (D.N.) spontaneous nystagmus upon eye-closure and in patients without spontaneous nystagmus (remain. pat.).

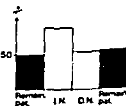


Fig. 6. Number of late functional ... tients with increasing (I.N.) and decreasing ... taneous nystagmus upon eye-closure and ... without spontaneous nystagmus (remain. pat.).

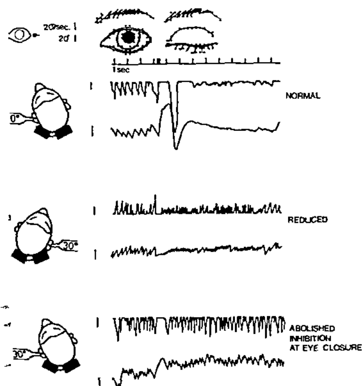


Fig 7 Various degrees of inhibition of calorically induced nystagmus by eye-closure

quency 72 patients in the material had taneous nystagmus In 34 cases of these) there was an increase in nystagmus and ases (21%) there was a decrease in nys ius upon eye closure

relations Patients who had a decrease ontaneous nystagmus upon eye closure latively few early functional symptoms icance almost at a 5% level see Fig 5) ersely spontaneous nystagmus increas pon eye closure was found in patients many and especially late functional symp (significance at a 1% level) (Fig 6)

rpretation The intensity of nystagmus eye closure may thus be assumed to re nervousness in the patient This correla nay seem difficult to explain It is well n however that sleepiness reduces nys is (Collins & Guedry 1962) It is also n that eye closure enhances sleepiness is clearly reflected by the increase of rhythm in the EEG The decrease of gmus upon eye closure in normals could then be an effect of sleepiness provoked by eye closure In nervous patients suffering from anxiety and irritability an increased alert ness may be expected In these patients eye closure may then *not* convey the normal de crease in alertness allowing the nystagmus to stay unaffected by eye closure

In this behaviour spontaneous nystagmus upon eye closure seems to reflect personality and may help to differentiate between func

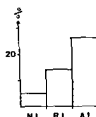


Fig 8 Number of patients in % with persisting nystagmus in light distributed in the groups with different inhibition of nystagmus upon eye-closure N I =Normal R I =Reduced and A I =Abolished inhibition by eye-closure

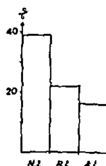


Fig 9 Number of patients in % with undulating eye movements upon eye closure distributed in the different groups of inhibition of nystagmus upon eye-closure N I = Normal R I = Reduced and A I = Abolished inhibition by eye-closure

tional and other sources of vertigo and dizziness

III *Information by Caloric Nystagmus Responses*

1 *Difference in caloric reactivity*

Appearance The intensity of the caloric response was measured by maximum speed of the slow component A difference between right and left ear exceeding 20% was, according to Jongkees & Philipszoon (1964), regarded significant

Frequency 87 patients showed a difference in caloric response exceeding 20%

Correlations These 87 patients did not differ much with respect to other signs and symptoms from patients with equal caloric responses There was no correlation to one sided loss of hearing to a spinning sensation outside head nor to other signs or symptoms indicating a labyrinthine disorder On the other hand of the 17 confirmed Meniere cases 59% showed a difference in reactivity against 24% among other patients

Interpretation Thus even if unequal caloric responses are frequently found among Meniere cases the solitary finding of a difference in caloric reactivity does not significantly indicate this disease It may then be concluded that clinical information derived from the solitary finding of a difference in caloric response so often assiduously looked for must be regarded as rather overestimated

2 *Persistence of caloric nys[illegible] at eye closure*

Appearance Normally vesti[illegible] is distinctly inhibited by eye-c[illegible] strom 1973) However this i[illegible] loric nystagmus seems however tients to be moderate or even [illegible] missing (Fig 7)

Frequency In most patients [illegible] tients this normal inhibition [illegible] 104 patients there was a decre[illegible] mus—although quite moderate— tients there was little or no cha[illegible] nystagmus upon eye-closure

Correlations Caloric nystag[illegible] upon eye closure also persisted fixation in light (Fig 8) (1% sig[illegible] rarely appeared in patients wi[illegible] (Fig 9)

Patients in this group with pers[illegible] mus upon eye closure also co[illegible] large number of functional sy[illegible] had been present even before th[illegible] tigo (Fig 10) The difference in from the group with normal i[illegible] loric nystagmus upon eye closu[illegible] cant at a 1% level

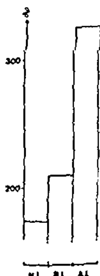


Fig 10 Number of early functional [illegible] tributed in the different groups of i[illegible] upon eye-closure N I = Normal R I [illegible] A I = Abolished inhibition by eye-closure

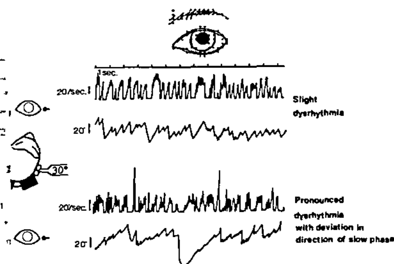


Fig 11 Different degrees of dysrhythmia and deviation of the eyes in direction of slow phase during caloric test in two different subjects

rpretation According to Demanez & ix (1969) the persistence of caloric nys-s in light indicates a central disturbance due to the positive correlation, persist-stagmus at eye closure should also be an of central disturbance

lack of undulations among these pa-s supports the assumption of some kind of al among patients in this group Such an al seems to preserve the nystagmus by eracting the sleepiness conveyed by eye-re by the same mechanism as discussed pontaneous nystagmus upon eye closure is assumption of a nervous arousal or ness is further supported by the finding of ge number of functional symptoms—anx nervousness and irritability Along with symptoms can be expected a tension ing the basis for the arousal In this way ne three correlations may be explained g the same lines Anxious patients have her level of arousal making expressions leepiness undulations rare and allowing agmus to persist in spite of eye closure

ysrhythmia in the caloric nystagmus onse

earance An adequate and practical defini-of dysrhythmia seems still to be missing degree of dysrhythmia had then to be sub vely evaluated Examples of slight and pronounced dysrhythmia in the caloric test are presented in Fig 11

Frequency 88 patients had a slight and 8 a pronounced dysrhythmia in the caloric nystagmus response

Correlations Most patients in the pronounced dysrhythmia group were found in the highest age decade (71–80 years) (Fig 12) Patients with dysrhythmia in the caloric nystagmus response complained of a large number of early functional symptoms (Fig 13) The difference vis a vis other patients was significant at a 5% level No differences were found with respect to late functional symptoms (Fig 14)

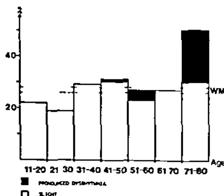


Fig 12 Number of patients in % with pronounced and slight dysrhythmia within the different decades The two lines in the figure represent the two types of dysrhythmia in the whole material

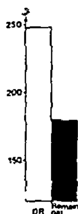


Fig 13 Number of early functional symptoms in % in patients with dysrhythmia (DR) and without dysrhytmia (remain pat)

Interpretation An assumption that dys rhythmia expresses disturbances in central in tegration essentially on a vascular basis (Hen riksson et al , 1972) is supported by the fact that dysrhythmia was so frequent among the oldest patients The clinical value of this, how ever, is diminished by the fact that also early *functional symptoms seem to correlate to dys-* rhythmia

COMMENT

Spontaneous nystagmus may be the sign most frequently looked for in the nystagmogram This finding, although frequently met with in our recordings, presented unexpectedly few correlations to other signs and symptoms Thus nystagmus seems to appear just as fre- quently in peripheral as in central disorders

The negative correlation between the pres ence of spontaneous nystagmus and presence of undulations has not been reported earlier and may be of no great clinical interest It does confirm, however, the concept that the inten sity of spontaneous nystagmus is expressing alertness and that the presence of undulations expresses sleepiness

The difference in caloric reactivity between the two ears, a sign very assiduously sought by many otologists showed no significant relations, whether to unilateral hea[illegible] vertigo lasting for 2-5 hours or t[illegible] functional symptoms which m[illegible] expected This indicates that th[illegible] finding of a difference in calor[illegible] from clinical point of view ma[illegible] somewhat overestimated earl[illegible]

Thus, even if the otologist m[illegible] be disappointed when looking f[illegible] cating a peripheral lesion many f[illegible] nystagmogram seem to be of use[illegible] evaluation of the cases by suppo[illegible] diagnosis

A positive tracking test althou[illegible] specific, gives weighty support to [illegible] disturbance as being responsible f[illegible] tigo The regular but patholog[illegible] the tracking test replacing the n[illegible] flexible movements seem to be the [illegible] most relevant finding in the whole [illegible] graphic test battery This sign of [illegible] saccades was present in all 5 seri[illegible] the material

Saccades instead of smooth f[illegible] ments gaze nystagmus and pers[illegible] tagmus in light are all findings th[illegible] interpreted as expressions of ce[illegible] ances The obvious connection bet[illegible] pathological saccades and gaze ny[illegible] the one hand and also between the[illegible] and persistence of nystagmus in l[illegible] this assumption

The various objective findings in t[illegible] mogram connected with early and l[illegible] tional symptoms also seemed to be [illegible]

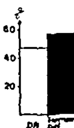


Fig 14 Number of late functional sy[illegible] tients with dysrhythm a (DR) and [illegible] rhythmia (remain pat)

In patients with early functional ms the caloric nystagmus was dysic and also persisted in spite of eye The vertigo in these cases was very it against treatment

atients with late functional symptoms, neous nystagmus when present tended ease at eye-closure The prognosis with t to vertigo in these cases was relatively

differences between the two different s of symptoms connected with early te functional symptoms also indicated ng pathology in such cases

conclude, even if the nystagmogram not often directly inform about labyrindisorder, a lack of signs indicating centurbances may support such a diag- However, a long row of signs in the gmogram would seem to reveal central bances possibly related to vertigo

ZUSAMMENFASSUNG

ntersuchung betraf 338 Patienten mit Schwindelge- o es darauf ankam nystagmische Befunde zu inter n Um periphere Erkrankungen des Vestibularis- tes zu erfassen sind Symptome wie Spontanny s Lagenystagmus und Unterschiede in der kalo- Erregbarkeit von begrenztem Interesse Zentrale zen kann man dagegen erfassen indem man ein nogen zur Blickfolge eines beweglichen Zieles mit rscheinen einer irregularen oder Saccaden ahn Nystagmusreaktion oder ein Bestehen einer Ny sreaktion im Licht und beim Augenschließen oder Zunahme des Spontannystagmus beim Augen en oder bei einer dysrhythmischen Reaktion im chen Test findet Patienten mit psychischem unterscheiden sich von Schwindelpa anderen Ursprungs durch ihre Zunahme des Spon tagmus (wenn vorhanden) oder bei dem Bestehen Jonischem Nystagmus beim Augenschließen

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NASAL MUCOCILIARY FUNCTION DURING PENICILLIN TREATMENT

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Abstract Nasal mucociliary function was studied in 21 healthy subjects before during and after penicillin administration No change in the mucociliary function occurred during penicillin treatment Nor was any difference observed between men and women or between smokers and non smokers On the other hand the measurements revealed a significantly smaller intra individual than inter individual variation As the epithelia of nasal cavity and Eustachian tube are morphologically of the same type, it is concluded that the increased incidence of serous otitis media which has been observed after the introduction of penicillin in the treatment of acute otitis media can scarcely be due to an inhibitory effect of penicillin on the mucociliary function of the Eustachian tube

-vitro studies have shown that penicillin erts an inhibitory action on the ciliary function This was demonstrated by Proetz as early as 1945 in ciliated respiratory epithelium removed from the tracheal mucosa of rabbits The effect was related to the concentration Thus, a solution of 5000 I U of penicillin per ml reduced the ciliary movements to one-half within a few minutes, whereas a solution of 12 5 I U per ml was without any effect Strong & Tonkin (1951) who used human mucosa from the maxillary sinus removed during operation for sinusitis demonstrated that the ciliary movements ceased after 2 minutes when the mucosa was rinsed in a penicillin solution containing 300000 I U per ml, and that the effect was reversible In recent in-vitro studies on the mucociliary function of the rabbit trachea Toremalm et al (1977) likewise demonstrated that a penicillin solution exerted a pronounced inhibitory effect on the ciliary activity resulting in ciliestasis within ½–2 hours, but they did not report the penicillin concentration used O hand, a 10% gentamycin solution b on the ciliary activity

If this effect of penicillin on the thelium of the Eustachian tube is in vivo, this might possibly exp creased incidence of serous ot has been observed during the la and which seems often to have oc preceding penicillin therapy for media (Kokko, 1974)

The purpose of the present st clearance in healthy subjects was whether penicillin in therapeutic inhibitory effect on the mucocilia vivo It must be assumed that the the mucosae of the Eustachian nasal cavity are identical, since the the Eustachian tube is morpholog same type as that of the resp (Sadé, 1966, Hentzer, 1973)

MATERIAL AND METH

The mucociliary function was st saccharin/sky blue technique d Andersen et al (1974*a*) A blue charin particle, 0 5–1 0 mm in on the superior surface of the in about 0 5 cm behind its anterior 4–5 cm from the apex of the nose is then instructed to swallow on minute, and the time lapse un strong sweet taste is recorded passage of the particle is verified by ing of the mucosa of the nasoph

I *Nasal clearance before during and after administration of penicillin*

ex	Age (yrs)	Nasal clearance relative to penicillin administration Before (min)			During (min)			After (min)			Serum conc (µg/ml)
	27	7 5	7 0	10 0	10 5	9 0	9 0	8 5	11 0	8 0	6 9
♂	28	14 0	8 5	17 0	8 0	15 0	6 0	11 0	6 0	9 0	10 2
♂	24	11 0	11 0	13 0	10 0	8 5	9 5	11 0	10 0	12 0	7 8
♂	24	7 5	6 5	8 0	14 0	14 0	12 5	12 0	1? 0	11.5	7 6
♂	25	–	7 5	9 0	7 5	7 0	–	7 0	10 0	14 0	17.2
♂	26	13 0	10 0	11 0	9 0	7 5	9 0	9 0	10 0	10 0	9 3
♂	28	–	10 0	9 0	12 0	9 5	7 5	9 0	11 0	9 5	8 9
♂	27	12 0	10 0	14 0	11 0	8 0		14 0	9 0	10 0	7 2
♂	24	18 0	7 0	20 0	10 0	11 5	7 5	8 5	18 5	9 5	8 9
♂	25	8 5	6 0	6 5	6 5	8 5	9 5	8 0	6 5	11 0	21 5
♂	26	8 0	9 5	9 5	7 0	8 0	6 0	7 5	8 5	–	18 2
♂	24	8 0	7 5	4 0	7 0	7 0	7 0	7 5	7 5	6 5	8 5
♂	24	15 1	11 0	10 0	12 0	11 5	11 0	9 0	–	7 0	15 0
♂	26	17 5	9 5	9 0	8 0	12 0	9 0	–	8 0	6 0	14 5
♂	23	8 5	9 0	8 0	7 5	7 0	7 5	7 0	8 0	8 5	4 7
♀	24	10 5	12 0	11 0	10 5	7 5	9.5	9 0	11 0	7 5	15 6
♀	27	11 5	9 0	11 0	11 0	10 5	9 5	12 0	9 5	10 0	28 3
♀	25	–	8 0	9 5	9 5	8 5	8 5	7 5	10 0	8 5	4 9
♀	21	7 0	8 0	13 0	9 5	10 5	13 5	11 0	9 0	7 0	11 2
♀	19	18 0	12 0	11 5	17 5	12 0	16 0	9 0	10 0	9 5	10 3
♀	37	8 0	8 5	6 0	7 5	6 5	8 0	9 5	7 5	7 5	19 5
e		10 1 min			9 5 min			9 3 min			12 2

e of 181 measurements 9 6 min
e for men 9 4 min for women 9 9 min
e for smokers 9 9 min for non smokers 9 5 min
studies 7 were unsuccessful ( ) one not performed ( ) nos 1 6 8 and 20 excluded (see text)

y simple and easy method for the meas ent of mucociliary nasal clearance As n by Andersen et al (1974*a*) there is a ficant correlation between this method the more complicated technique with active particles

e subjects studied were 25 healthy cal students and nurses, of whom none had any respiratory tract infection during preceding 3 weeks Before each measure t the subject concerned was acclimatized staying in a stable enviroment for at least ur Our studies extended over the period March 1 to May 5 1977 Twice during experimental period the temperature and ive humidity in our laboratory were re ed continuously for a week The room perature was a constant 21–23°C and the idity content of the air was 30–35% Stud eported by Andersen et al (1972, 1974*b*) Bang et al (1967) have shown that changes in temperature and relative humidity of the order just mentioned do not have any effect on human nasal mucociliary function

Each subject was studied on nine occasions, distributed in three series, viz (1) meas urements were performed on three occasions before the administration of penicillin, (2) on three consecutive days during penicillin treat ment, and (3) three times after this treatment The penicillin was given by mouth in the form of three tablets, each of 1 million I U of phenoxymethylpenicillin (Fenoxillin®, Novo) in the morning and evening for 3 days The measurements of nasal clearance were per formed 1½ hours after the morning dose and ½–1 hour after the presumed maximum serum concentration (Bergan et al , 1975) On the second day of penicillin treatment the serum concentration of penicillin was determined simultaneously with the clearance measure ment

RESULTS

Of the 25 subjects, four (nos 1, 6, 8 and 20) were excluded because of a nasal clearance time of more than 20 minutes on two consecutive measurements in the first series Subject no 1 had a few nasal crusts, but otherwise no discomfort No 6 had had sinusitis 6 weeks previously, and swelling and redness of the conchae were still present Nos 8 and 20 were objectively normal and had no discomfort The remaining 21 subjects were 11 men and 10 women whose ages ranged from 19 to 37 (average 25 4) years Six subjects (nos 2, 3, 4, 11, 17 and 22) were smokers, stating that their average consumption of tobacco was 4 cigarettes daily

The average nasal clearance time was 10 1 min before, 9 5 min during, and 9 3 min after the administration of penicillin These values are not significantly different Nor was any difference found between men and women or between smokers and non-smokers, but the intra-individual difference of the measurements was significantly smaller than the inter-individual one ($p < 0.05$)

The average serum concentration of penicillin was 12 2 µg/ml (range, 4 7–28 3 µg/ml) The penicillin treatment resulted in diarrhoea in 6 subjects (28 6%), in one (no 11) so severe that penicillin had to be withdrawn after 2 days

CONCLUSION

Our study shows that penicillin administered orally to healthy subjects even in relatively large therapeutic doses does not have any effect on nasal mucociliary function It is therefore assumed that penicillin does not have any effect on the ciliary function of the Eustachian tube either because its mucosa morphologically is of the same type as that of the nasal cavity The increased incidence of serous otitis media observed after the introduction of penicillin for the treatment of acute otitis media can therefore scarcely be explained by an inhibitory effect [illegible] the ciliary function of the Eu[illegible]

ZUSAMMENFASSUNG

Die mukoziliare Funktion in der Nase [illegible] und nach Penicillinbehandlung bei 21 [illegible] personen untersucht Keine Änd[illegible] Funktion wurde wahrend der Pen[illegible] achtet Auch zeigte sich kein [illegible] Mannern und Frauen oder zwischen [illegible] Nichtrauchern Dagegen zeigten [illegible] sungen daß die intra individuelle [illegible] kleiner war als die inter individuelle [illegible] der Nasenhöhle und der Ohrtrompete [illegible] selben Typ ist wird konkludiert, daß [illegible] denz von seröser Mittelohren[illegible] Einfuhrung von Penicillin in der Be[illegible] Mittelohrentzundung festgestellt werden [illegible] einen hemmenden Effekt des Penicillins [illegible] ziliare Funktion in der Ohrtrompete [illegible]

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.MUNOGLOBULINS IN NASAL SECRETIONS AND NASAL MUCOSA IN PERENNIAL RHINITIS

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(Received July 5 1977)

Quantitative identification of albumin and im ›ulin G A M and E in the nasal secretion and ›s done in 25 patients with perennial rhinitis and ›nal subjects By immunofluorescent microscopy ›es from the inferior turbinate immunoglobulin ›g plasma cells were identified by using FITC ›nt globulin against IgG IgA IgM and IgE The of IgG and IgA in the nasal secretion and serum significant and there was no difference between ›nd patient groups IgM was detected in nasal › in the patient group probably due to increased ›ility of the mucosa This explanation is supported ›act that albumen was also found in a rather high ›ation in the patient material The presence of ducing plasma cells was more frequently found in ›nt group than in the control subjects and in those › where presence of these cells was proved a oncentration of IgM in serum was shown pos ›dicating a generally increased IgM activity This › the case with IgE Furthermore increased IgE nasal secretion and serum was found in the patient Identification of antigen specific IgE (RAST) in cretion gave poorly reproducible and uncertain re ›ich could not be correlated with the results from ›g of serum

g the past 10 years the role played by IgE pathogenesis of allergic diseases has the subject of extensive research The ›urement of total IgE (RIST) and allergen ›ic IgE immunoglobulins (RAST) in se ›as been of great importance in the dif ›tial diagnosis of asthma and atopic der ›is (Johansson et al 1972) The immuno- ›lins in nasal secretions have also been ›ed in asthma hayfever and polypi nasi ›day et al 1971 Younginger & Gleich Mygind et al 1975) and the correlation cutaneous nasal and inhalation tests has studied by Houri et al (1972)
E producing plasma cells were detected in nasal mucosa of normal individuals for the first time by Tada & Ishizaka (1970), com pared with the number of IgA and IgG pro- ducing plasma cells, however, they occur very sparsely (Mogi, 1975) No significant differ ences in the distribution of IgA, IgG and IgM in the nasal mucosa in normal and allergic pa tients have been found (Brandtzaeg et al, 1967), and the distribution of IgE producing plasma cells in the nasal mucosa and polypi nasi in atopic patients has not been significant (Pesak, 1971, Bass et al, 1974)

Perennial rhinitis presents considerable dif- ficulties in differential diagnosis With the methods of testing commonly used in allergo- logy it is often not possible to identify a spe cific allergen, and these cases are often at tributed to a vasomotor unspecific instability We have therefore found it helpful to examine the occurrence of immunoglobulins in nasal secretions in patients with perennial rhinitis, particularly by screening for allergen specific IgE antibodies, compared with the level in normal subjects A fluorescent microscopic study has been done of immunoglobulin pro- ducing plasma cells in biopsies from the in ferior turbinate

MATERIAL AND METHOD

Twenty five patients with perennial rhinitis of at least 2 years' duration were included in the trial The history included running nose, sneezing and blockage but without seasonal variation

Table I *Albumin and immunoglobulin concentrations in serum and nasal secre-* ... *normals (N-1 to N-15) and 25 patients (P-1 to P-25) with perennial rhinitis*

	Serum					Nasal secretion			
	Alb (g/l)	IgG (U/ml)	IgA (U/ml)	IgM (U/ml)	IgE (U/ml)	Alb (g/l)	IgG (U/ml)	IgA (U/ml)	IgM (U-
N 1	45	126	133	192	70	4.2	4.7	13.5	6.6
N 2	43	114	65	83	40	4.2	2.4	8.4	0
N 3	46	88	78	109	100	4.2	1.5	8.4	0
N-4	48	102	70	127	0	2.4	0.9	8.4	0
N 5	38	90	85	66	0	5.1	1.2	6.0	0
N-6	45	152	85	213	0	3.0	3.6	9.9	0
N 7	41	127	106	37	0	3.3	2.1	8.4	0
N 8	38	102	95	71	30	4.3	4.8	16.8	0
N 9	42	176	133	50	0	4.5	4.5	12.9	0
N 10	36	88	151	144	0	3.3	2.1	10.2	0
N 11	47	109	111	81	120	3.0	2.7	14.4	0
N 12	39	177	103	297	40	4.8	3.9	14.4	0
N 13	40	123	119	207	0	3.3	4.2	12.0	0
N 14	44	151	71	99	0	1.5	8.7	32.1	0
N 15	38	102	103	126	30	0.3	0.9	8.7	0
P 1	47	123	59	156	340	5.1	7.5	19.8	8.4
P 2	44	102	67	189	160	2.7	2.4	12.0	0
P 3	47	102	78	244	130	1.5	3.0	18.0	0
P-4	41	113	125	97	250	11.4	32.4	41.4	13.8
P 5	42	177	94	104	220	5.1	2.7	19.8	0
P-6	43	90	67	156	60	7.8	2.1	9.3	0
P 7	44	137	125	280	100	6.9	3.3	10.8	0
P-8	41	102	67	207	800	4.8	1.5	8.1	0
P 9	46	137	157	104	0	2.7	2.4	21.3	0
	49	190	181	112	140	4.8	9.9	15.6	0
	45	91	117	97	0	6.6	1.2	4.5	0
	45	158	86	187	150	6.9	3.0	7.5	0
	46	123	133	97	210	5.7	3.6	14.1	0
4	47	202	71	71	100	5.4	1.2	10.2	0
P 15	44	151	23	99	160	3.9	6.8	23.1	0
P 16	43	137	139	126	10	1.5	0.9	4.5	0
P 17	43	158	101	140	0	6.0	1.2	13.2	0
P 18	48	113	139	112	280	11.1	20.9	28.8	12.9
P 19	45	113	94	120	170	4.2	1.5	11.1	0
P 20	41	145	94	199	150	1.8	3.9	27.0	0
P 21	48	179	0	213	10	6.6	10.5	2.7	15.9
P 22	50	126	150	39	120	5.7	2.4	10.2	5
P 23	47	114	106	101	4 000	9.3	11.7	13.5	6.6
P 24	50	114	114	90	40	4.5	3.0	6.3	0
P 25	45	166	146	109	160	7.5	1.8	8.4	0

Prior to inclusion in the trial a standard ENT examination was done including X ray of the nasal sinuses. Patients with polypi nasi causing obstruction with active sinusitis and patients requiring treatment for asthma were excluded from the trial as also were individuals under 15 years of age.

The control subjects were 15 volunteers with no history of nasal disease or allergic manifestations.

Investigations for eosinophils in the nasal secretion as well as prick test for ... antigen were done.

Examination of nasal secretion

The nasal secretion was taken ... with filter paper strips (Whatman ... described by Lorin et al (1972) and ... et al (1975). Phosphate buffer ... (Coon's buffer, pH 7.1), so that the ... was approximately 1 : 2, and the ... factor was established accord...

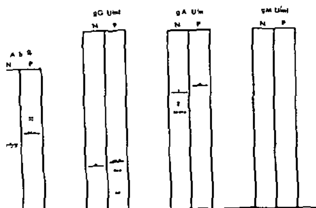


Fig 1 Diagram showing albumin levels as well as IgG IgA and IgM in nasal secret on *N* normal material *P* patient material

tion was done by centrifuging for 10 es at 15 G in a 5 ml plastic syringe with forated base fitting into a plastic centri g glass After passing through a Millipore about 2 ml clear eluate was obtained was discarded if there was a reaction to l after testing with Hemastix test paper e level of albumin was measured by using modified electro-immunoassay technique rell 1966) and the amounts of IgG IgA IgM in serum and nasal secret on were ured by a modified electro-immuno tech by carbamylating the samples prior to rophoresis (Weeke 1968) Total IgE lev ere measured by the radioimmunosorbent nique (RIST) with Phadebas® IgE kit and gen specific IgE antibodies against 13 al ns were identified by using the Phadebas® T analysis system

mination of serum

centration of albumin IgG IgA IgM and as well as RAST screening were done in same way on serum from the patient ma l and the control subjects

unofl iorescent investigation

ler local anaesthesia biopsies were taken the inferior turbinate placed in AMES T compound and frozen to −20°C Im nofluorescent microscopy was performed as described by Crabbe et al (1965) using a direct technique in a 4 μm thick piece of tissue by conjugating with FITC marked rabbit antihuman gamma globulin (Behringwerke) di rected at IgG IgA IgM and IgE After wash ing off of surplus antibodies the specimens were studied under a Leitz fluorescent micro-scope The results were controlled by blocking tests with corresponding unmarked antisera The blocks were fixed in paraffin wax and methylgreen–pyronin stained specimens were produced for assessment of the number of plasma cells in the biopsies

RESULTS

The levels of albumin and IgG IgA IgM and IgE in serum and in the nasal secretions of patients and controls are shown in Table I and Figs 1 and 2

The comparative mean values and in cases where the material could not be proved of normal distribution the medians are shown in Table II From this it appears that contents of albumin IgM and IgE in the nasal secretions are significantly higher in the patient material than in the controls

In serum higher albumin and IgE levels were found in the patient group

Five patients showed a reaction to prick tests to various foods (fruit meat fish) as well

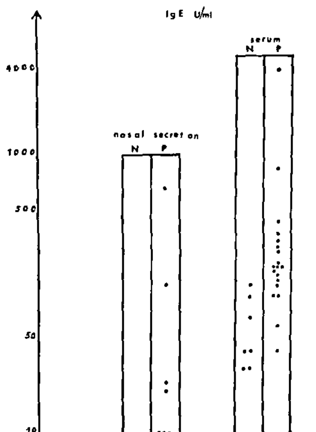


Fig 2 Diagram showing levels of IgE in nasal secretion d serum *N*=normal material *P*=patient material

to pets (dog, horse) Half of the patients did not show nasal eosinophilia and only a few showed eosinophilia to a marked degree

No correlation between these findings and the immunoglobulin levels in serum and secretions was found

RAST-screening to 13 different in nasal secretions resulted in proved positive reactions (in class 2) sults could not be related to the co values obtained by RAST analysis and no similarities between prick RAST analysis in serum and nasal were found

As the results were difficult to in other investigation of the RAST serum and secretions was done months later in 1/4 of the patients secretion values found were still un there was no reliable agreement with ously found values

In the biopsies a number of artefac found in the methylgreen-pyronin c which were due to the preparation a did not permit any actual histological tion The number of plasma cells w scanty and in some few specimens th ence of plasma cells could not be strated

The presence of IgG and IgA fluo plasma cells was not notable and t peared equally in the control group a patient group IgM fluorescent plas were seen in 7 patients, but only in on control subjects IgE fluorescent cel found rather frequently, i e in 9 patie 4 controls In nearly all instances th found in isolation (Fig 3) and only

Table II *Statistical evaluation of albumin and immunoglobulin concentrations in se secretion*

Where a normal distribution could be assumed mean value *x* is stated and Student s test has been used mainder the median is stated and statistical analysis has been done by Mann Whitney s test P pat N control material *p* confidence limits U international units

	Serum					Nasal secretion				
	Alb (g/l) X	IgG (U/ml) X	IgA (U/ml) X	IgM (U/ml) X	IgE (U/ml) median	Alb (g/l) X	IgG (U/ml) median	IgA (U/ml) X	IgM (U/ml) median	[illegible]
N	42 0	121 8	100 5	126 8	0–10	3 4	2 7	12 3	–*	[illegible]
P	45 2	134 5	101 5	138 0	150	5 6	3 0	14 4	–*	
Diff	3 2	12 7	1 0	11 2		2 2		2 1		
$p<$	0 003				0 01	0 004			0 10	[illegible]

* Median lower than measurement sensibility of laboratory analysis



Solitary IgE producing plasma cell Immunofluo-microscopy of biopsy from inferior turbinate in al rhinitis

t were several fluorescent cells found)

re was no correlation between the pres- of plasma cells and the concentration of noglobulin fractions in the nasal secre- By investigating the relation between esence of fluorescent plasma cells and noglobulin concentration in serum, both and IgE fluorescent plasma cells were in patients with considerably higher se- oncentration of the comparable immuno lin (IgM $0.02>p>0.01$, IgE $p<0.1$)

DISCUSSION

titative identification of protein in nasal tion has only been used a few times since et al (1972) found a method for this, d on weighing the secretion picked up on paper strips In previous investigations mmunoglobulin concentration was meas- in relation to the concentration of albu in nasal wash out fluid, which has been re- ed as a rather constant factor As shown lygind et al (1975) the concentration of min varies considerably, and in pathological conditions in the nose, the concentration will increase, as the nasal mucosa becomes permeable to protein, a fact which we also found in our investigation

This leakage through the mucosa is confirmed by the fact that some patients with high albumin concentrations (P4, P18 and P23) had also a very high concentration of a number of immunoglobulins in their secretion We are not able to explain why we found a higher serum-albumin concentration in the patient group than in the normal material, but the difference was statistically significant ($p<0.003$)

The ratio of $IgG_{secretion}/IgG_{serum}$ and $IgA_{secretion}/IgA_{serum}$ was identical in both the patient group and controls, which indicates that no change in the local production of these globulins is found in perennial rhinitis That the ratio $IgA_{secretion}/IgG_{secretion}$ is approximately 7 times larger than the ratio IgA_{serum}/IgG_{serum} is due partly to the fact that IgA is produced locally to a considerable degree, and is also secreted actively through the glands of the nasal mucosa, as two IgA moleculars are linked with a "secretory piece" (Brandtzaeg, 1973) In the microscopic investigation of the mucosa we have found a number of plasma cells along the bottom of the gland acini

One patient (P21) had extremely low concentrations of IgA in both serum and nasal secretion This was a 24-year old male patient who had no unusual history in comparison with the others His nasal symptoms were moderate, and he had no history of immunological deficiency All other examinations were negative

The concentration of IgM in nasal secretions has previously been investigated on a few occasions Under normal circumstances it is not excreted in nasal secretions (Lorin et al , 1972), a fact which is shown in our control subjects Probably due to the leakage mentioned above, we have in the patient group shown concentration of IgM in secretion, the difference being of low significance ($p<0.1$) IgM producing plasma cells were found in 1/3 of the patients, and it is notable that these had



Fig 4 IgE produc ng | gathered in groups In rescent microscopy of inferior turbinate in pe rhinitis

significantly higher serum concentrations of IgM than the others (0 02>p>0 01) This could be an indication of a generally increased ...vity within the IgM plasma cell population the body There was no proof that this in...ased IgM activity could be due to some sort of compensation for a poorly developed IgA system, as these patients did not differ from the others with regard to concentration of IgA in serum or secretion

Concentration of IgE was found increased in serum (p<0 01) and nasal secretion (p<0 1) in the patient group Corresponding changes were shown in perennial rhinitis by Handrock & Baumgartel (1975) IgE-producing plasma cells in larger amounts have not been shown in the patient group and the controls, but as was the case with IgM there was a tendency for a higher concentration of IgE in serum in patients where IgE fluorescent plasma cells were present than in the others (p<0 1), which may indicate a general increase of IgE plasma cell population in perennial rhinitis

Identification of allergen specific IgE antibodies (RAST) in serum is of limited interest in perennial rhinitis, probably due to the small size of the target organ One could hope that RAST investigation of ... secretion could be of great value in ... ing in which patients the allergic me... are of importance (allergic rhinitis) ... which patients an unspecific, vaso... stability is of importance (vasomotor ...

Huggins & Brostoff (1975) have ... group of patients with positive nasal ... tion tests and specific antibodies in ... secretion against house dust mite ... negative prick test and negative RA... rum In our investigation by RAST ... of nasal secretions, we, however, ... poorly reproducible and weak reactio... could not be interpreted satisfacto... same result has been reported by H... Levy (1975)

ACKNOWLEDGEMENTS

We should like to extend our thanks to the Ce... ratory at Aalborg Sygehus Syd for very ... in the laboratory investigations The immuno... microscopic investigations were done at the ... Institute at Aalborg Sygehus Nord Pharma... plied us with kits and helped with the total ... specific IgE localization

ZUSAMMENFASSUNG

ative Bestimmung von Album n Immunglobulin

asmazellen bestimmen Das Vorhandensein des id IgA im Nasensekret und im Serum bot nichts ichten dar und es bestand kein Unterschied en der Normalgruppe und der Patientengruppe ar wahrscheinlich wegen der vergrößerten Perme der Schleimhaut im Nasensekret der Patienten nachweisbar Diese Annahme stutzt sich darauf bumin ebenfalls in wesentlich hoherer Konzentra ı Patientenmaterial vorgefunden wurde Das Vor isein der IgM produzierenden Plasmazellen war je iaufiger in der Patientengruppe als im Normalma und bei den Patienten bei denen diese Zellen nach en wurden fand man hohere Konzentration des n Serum als bei den ubrigen Patienten was auf ı d esen allgemein gesteigerte IgM Aktivitat deutet stimmung des antigenspezifischen IgE (RAST) im sekret ergab schwer reproduzierbare und unsichere ısse die auf die entsprechende Nachprufung des s nicht zuruckgefuhrt werden konnten

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THE NEUROMUSCULAR PHONATORY CONTROL SYSTEM AND VOCAL FUNCTION

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(Received July 14 1977)

Abstract This paper reports on recent studies supposed to answer the question whether there exists a relationship between the ability of kinesthetic voice control i e the operational efficiency of the neuromuscular phonatory control system and the quality of vocal function In three subject groups with differing qualities of vocal function (24 singers 33 vocally untrained persons and 30 patients with hyperfunctional dysphonia) pitch and intensity changes of the speaking and singing voice were studied during interruption of auditory feedback by increasing binaural masking (white noise of 60 80 100 and 110 dB SPL) by means of our recently developed fundamental frequency analyser The following results were obtained the increase in speech intensity level was similar in all groups but the elevation of mean speaking fundamental frequency differed significantly the dysphonic group reacted to binaural masking with the greatest pitch incre nts and the vocally trained group (singers) with the lest ones Additionally dysphonic patients showed nificant reduction in pitch variations (speech melody) oice accuracy i e the ability to reproduce a given reference tone under mask ng cond t ons

ighest incidence of an incorrect pitch regulation (' initial overswing) during sudden upward shifts of fundamental frequency From these observations and on the basis of physiological data about pitch and intensity control the following conclusions are drawn the neuromuscular phonatory control system works more efficiently in vocally trained persons than in vocally untrained ones or especially in dysphonic patients thus revealing that kinesthetic control ability is closely related to the quality of vocal function As this ability can be measured by observing pitch changes under masking conditions an objective evaluation of the individual vocal function by means of fundamental frequency analysis seems feasible

The production of a vocal sound requires properly coordinated movements of numerous muscles of the larynx, the vocal tract, the thoracic wall and the abdomen, the precision with which this coordination is achieved determines the quality of vocal function I· words, vocal function will be the be more precisely the activity of syn and antagonistic muscles is balanced phonation Any discoordination by e cessive or insufficient tension or relax synergists and antagonists or adjacent groups will impede the accurate tuning phonatory apparatus, not only deteri voice quality but also increasing vocal which may become the basic reason development of a dysfunctional voc order As the phonatory process is con by a nervous control circuit (Fig 1) the function depends entirely upon the oper efficiency of this system It is well know empirical evidence and experiment (Hanley & Steer, 1949, Atkinson 195 mann & Tegtmeier, 1961, Morgon et al Hanley & Harvey, 1965, Charlip & 1969, Klingholz et al , 1973) that an in tion of the main control loop i e the a feedback, causes various disturbing upon vocal performance However a automonitoring of vocal output is on part of the phonatory control system clearly shown by the fact that in spite pairment or loss of hearing an adult p continues to phonate and articulate normally, although several voice and s parameters like vocal pitch and int speech melody or speech velocity (Per Arnold 1970) become somewhat altered other control loop is represented by a co neuromuscular reflex system being co summarized under the term ' kinesth

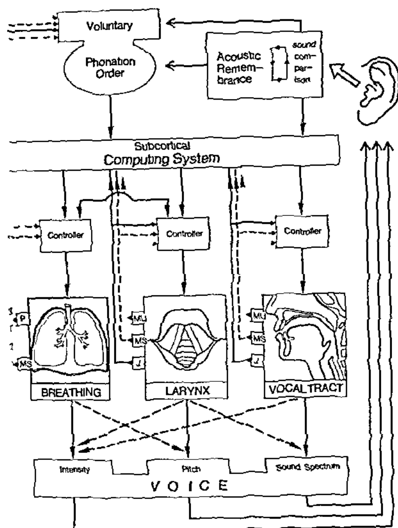


Fig 1 Schematic diagram of the phonatory control system *MU* mucosal mechanoreceptors *MS* = myotatic mechanoreceptors *J* = articular mechanoreceptors *P* pulmonary mechanoreceptors

" This system controls the motor action e phonatory process almost automatical ifferent types of mechanoreceptors in the ous membrane, the muscles and the joints ie larynx, *the thoracic and abdominal* and of the vocal tract monitor muscular ity and movements during phonation, the ent discharge from these receptors is fed to the motoneurone pools in the brain t operating as individual controllers for thing laryngeal action and vocal tract stment as well *as to an overriding sub* ical computing system which controls the e phonatory process according to the ntarily given phonation order (Fig 1) Al though this neuromuscular control circuit works entirely below the conscious level, kinesthetic feedback also reaches cortical regions to a certain extent where it is con sciously perceived as an overall idea of the present activity status of the phonatory apparatus (*one can feel' one's own voice*)

The neuromuscular reflex system has long been regarded as of minor importance for phonatory control when compared with the dominant role of *auditory feedback*, but sci entific work, especially that of Wyke and co-workers (Wyke, 1974*a*, *b*, Kirchner & Wyke, 1965, Kirchner & *Suzuki*, 1968) during the past decade strongly suggests that its opera-

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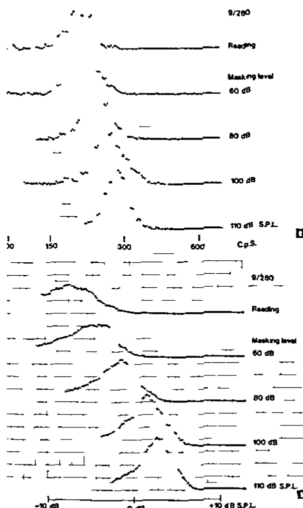


Fig 3 Increase in median fundamental frequency (A) and median intensity level (B) of the speaking voice due to increasing binaural masking (female subject) The median pitch and intensity levels are defined by the peaks of frequency and intensity histograms respectively Note the changing shape of histograms indicating a reduction in pitch (and intensity) variation

channel tape recorder (Revox A77) by of a contact microphone (Fig 2) For urement of the median pitch level the pitch range (i e difference between the st and the lowest fundamental frequen of a given sample) and the median sity level we used our fundamental fre y analyser (Fedders & Schultz Coulon which measures the fundamental fre y and the sound pressure level of a voice l continuously and simultaneously the signal of this analyser was fed into a l data processing system (DIDAC 800) h computed pitch and intensity distribu curves (histograms) for each sample In ion we directly recorded pitch and intensity curves of all singing voice samples on an UV light beam oscilloscope (Visicorder 2206) in order to obtain further information about tone stability the character of pitch variations and the precision of fundamental frequency shift from a lower to the next higher tone

2 *Results*

First we studied the changes of fundamental speech frequency and intensity under masking conditions The example of an individual analysis is shown in Fig 3 (A B) demonstrating that the individual reacts to the increase in masking noise level with rising elevation of both the median pitch and median intensity

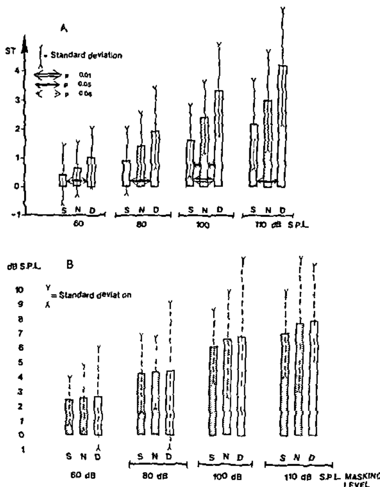
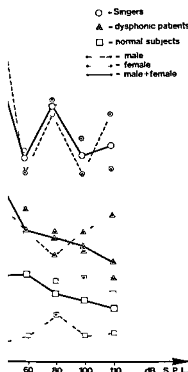


Fig 4 The average elevation speaking fundamental frequency median intensity level (B) at diffe ing noise levels *S*=singers vocally untrained subjects *D*= patients Statistically significant are indicated by arrows between

level (as defined by the peaks of the frequency and intensity histograms) This reaction known as Lombard Reflex (Lombard, 1911) was seen in all subjects tested for this study, but varied markedly with respect to the degree of pitch and intensity shift Consequently, average data calculated for each subject group showed wide standard deviations, but they revealed the interesting finding that the mean values of pitch elevation differed considerably between groups, whereas those of intensity increase did not (Fig 4 A, B) Singers elevated their fundamental speech frequency to a much lesser degree than the vocally untrained group, and the dysphonic patients showed the greatest frequency shift When analysed statistically, the differences in pitch elevation between groups turned out to be significant, as indicated in Fig 4 A (arrows between the columns in the diagram), whereas the slight differences between the mean values sity increase were not statistically sig

Speech melody, i e the variation damental speech frequency, is said come somewhat monotonous when ditory feedback is interrupted (Tr Winckel, 1957) In most cases we m same observation (for instance, in Fig decrease in pitch modulation is dem by the changing shape of the frequen grams with increasing intensity of t noise they become taller, steeper and more slender), but in some cases we a opposite reactions or no change modulation at all As a quantitative ex of pitch modulation we took the ratio base width to the height of a frequen gram and called it "modulation q Statistical analysis revealed that o speech melody was reduced in all the



ie average reduction in speaking pitch variation
creasing masking conditions *MQ* modulation
i e the ratio of the base width to the height
ency histogram the smaller the MQ the more
ous the voice

groups (Fig 5), but that this reduction could be confirmed statistically (beyond the 5% level of significance) for the dysphonic group only

The accuracy of tone reproduction under masking conditions was measured by comparing median pitch levels of phonated tones (as documented by frequency histograms, see Fig 6) with the fundamental frequencies of reference tones (from an electronic organ), the results of these measurements being summarized in the diagram of Fig 7 with and without binaural masking the singers showed the smallest (mean 0 21–0 29 semitones) and the dysphonic group the greatest (mean 0 46–0 75 semitones) pitch deviations from reference tones, the difference between the normal subjects and the dysphonic patients not being a very distinct one, however In singers, only a slight deterioration of voice accuracy can be noted with increased masking noise intensity, whereas in the vocally untrained and especially in the dysphonic group the median pitch deviation grows rapidly, reaching three-quarters of a semitone at a masking noise level of 110 dB SPL in both groups Statistical analysis disclosed that this deterioration of voice accuracy under masking conditions

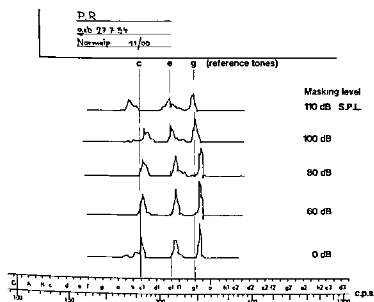


Fig 6 Individual analysis of the tone reproduction trial (female subject) Vertical lines indicate reference tones Median pitch levels of phonated tones are defined by peaks in the frequency histograms

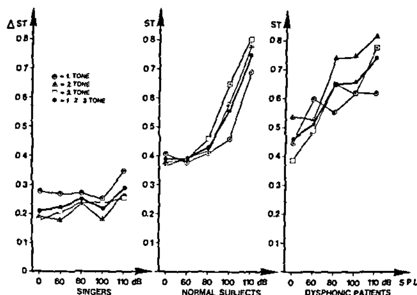


Fig 7 The dependence of [illegible] accuracy upon masking [illegible] mean frequency deviation of [illegible] reproduced tone from the reference tone *ST* = semitones

differed significantly between the singers and both the other groups (beyond the 5% level of significance), between normal and dysphonic persons no significant difference could be found, however

Regarding the influence of binaural masking [o]n tone stability the singers can no longer be [com]pared with the vocally untrained groups, all the singers sang with a regular modula[t]ion of fundamental frequency known as "vibrato", whereas all other persons showed the physiological irregular pitch fluctuations when sustaining a tone Most singers kept the frequency as well as the regularity of their vibrato unchanged in spite of being masked, only in a few cases did the vibrato be[come] somewhat irregular when the masking [noise] level reached 100 or 110 dB SPL all s[ingers] showed a more or less pronounced w[iden]ing of the frequency modulation ("vibrato swing"), which increased on average from [illegible] semitones (without masking) to 2 7 semitones (at a noise level of 110 dB SPL see Fig 8) In both the other groups we observed a s[light] —and statistically significant ($p<0\ 01$)— [wi]dening of the irregular frequency fluctuations upon increasing masking but the [most] remarkable finding was the highly significant ($p<0\ 01$) difference of tone stability between these two groups frequency histograms [illegible]

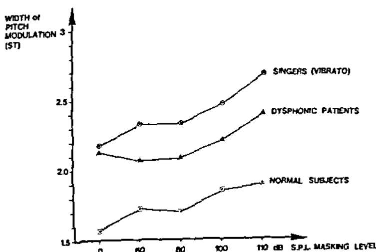
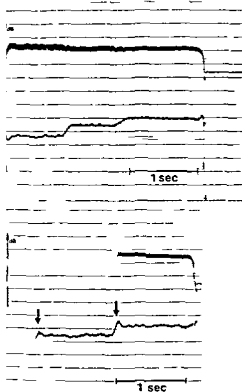


Fig 8 The widening of vibrato swing (singers) and of irregular pitch fluctuations (normal and dysphonic subjects) under increasing masking conditions Average [illegible] Note the marked difference between the normal and the dysphonic group *ST* = semitones



Fundamental frequency analysis of tone sequences y two d fferent subjects In (A) (female subject ; no se level 100 dB SPL) pitch is accurately the lower to the next higher tone whereas le subject in (B) (masking noise level 110 dB) an initial overswing of fundamental frequency) Hz c p s

a much wider range of pitch fluctua in dysphonic patients than in the normal on average this difference amounted to ximately 0 5 semitones

ditionally we would like to mention observation concerning the precision ch control when the fundamental fre is suddenly shifted from a lower to a r tone When reviewing pitch recordings e (singing) voice samples we noticed any subjects that they did not change to the next reference tone but im shifted vocal pitch upwards a little too (sometimes by more than two semi) thus having to lower it again in order ach the actually wanted tone (Fig 9B)

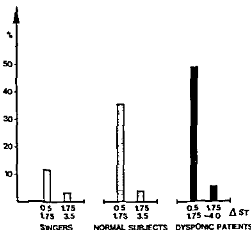


Fig 10 The incidence of initial overswing of fundamental frequency in percent [ΔST=difference (semitones) between the maximum pitch deviation of initial overswing and the median pitch of the entire tone]

The interesting point of this observation is the fact that the incidence of this phenomenon which we called initial overswing of fundamental frequency was found to be highest in the dysphonic patients and lowest in singers without showing any dependence upon the masking situation Fig 10 compares the incidence of initial overswing among all pitch changes recorded (ten pitch changes per person) The diagram not only reveals that inaccurate pitch changes most often occurred in the dysphonic group but also that initial overswing tended to be more pronounced in this group than in the other two

DISCUSSION

The vocal reaction in noise has to be regarded as a physiological reflex with the purpose of maintaining the possibility of verbal communication and at the same time, re-establishing acoustic automonitoring or—as Black (1950) put it—as the attempt of the human monitoring system to keep a normal experience at the speaker s ear This purpose could be achieved by an increase in vocal intensity only, not necessarily requiring a simultaneous elevation of vocal pitch however, for physiological reasons all people will more or less raise vocal

pitch when speaking with a louder voice As it is seen from our study there is no proportionality between increase in vocal intensity and pitch elevation some people raise their fundamental speech frequency in a noisy environment (or when being masked binaurally) much more than necessary, whereas others hardly change vocal pitch at all in spite of speaking markedly louder

With regard to pitch and intensity control, we know from several aerodynamic and electrophysiological studies (Faaborg-Anderson, 1957, Rubin, 1963, Isshiki, 1964, 1969, Faaborg-Anderson et al 1967, Hirano et al 1969, 1970, Perkins & Yanagihara, 1968, Koyama et al 1971, Gay et al 1972 and other authors) that, at least at low and middle pitches, intensity is controlled predominantly by aerodynamic forces, i e by the activity of the expiratory muscle groups, whereas pitch depends mainly upon tension, length and mass of the vocal cords, i e upon the activity of intrinsic and extrinsic laryngeal muscles the basis of this evidence, we have to con- that raising the fundamental speech needs a much higher activity of laryngeal muscles than intensity increments or that, in other words, speaking with a too high-pitched voice means considerably greater vocal strain than increasing vocal intensity Consequently, those people who tend to excessive pitch elevation in noise must be particularly predisposed to the development of dysphonia Briess (1957) suggested that "persons who raise their natural speaking voice will upset the dynamic equilibrium of the vocal muscles and develop voice difficulties" Brodnitz (1961) and Greene (1964) pointed out that heightened laryngeal muscle activity during phonation may be disruptive to optimal vocal function, and Dieroff & Siegert (1966) stressed the shift of fundamental speech frequency in a noisy environment as an etiological factor for the development of functional voice disorders A similar conclusion was made by Stone & Sharf (1973) on the basis of experimental data demonstrating that the voluntary use of atypical vocal pitch induced significantly more phonic voice changes than speaking at [illegible] intensity levels From this point of view cessive pitch increments accompanying increase in vocal intensity has to be reg[illegible] as the expression of a faulty and uneconom[illegible] regulation of the phonatary process [illegible] fore, our finding that the dysphonic pe[illegible] reacted to the interruption of auditory [illegible] back with the greatest shift and singers the smallest shift of fundamental sp[illegible] frequency strongly suggests that vocally trained persons and especially persons hyperfunctional dysphonia have a less cient neuromuscular phonatory control tem than have singers Obviously training over many years has taught singers always to limit muscular tension required minimum, thus enabling them without hearing their own voice, to keep the pitch level as much as possible wh[illegible] creasing vocal intensity

A flattening of the melodic accent [illegible] speaking voice is apparently a frequent companying phenomenon of pitch and [illegible] sity elevation during binaural masking [illegible] probably related to the increased tens[illegible] laryngeal muscles, because the group w[illegible] highest pitch increments, i e the dys[illegible] patients, also showed a more distinct tend[illegible] to reduce pitch variations than both the [illegible] groups It would therefore, seem reason[illegible] take an excessive reduction of pitch vari[illegible] under masking conditions as further [illegible] an uneconomically operating neurom[illegible] control system

Other evidence for the relationship be[illegible] kinesthetic control ability and vocal func[illegible] given by the results of our tone reprod[illegible] trials voice accuracy during [illegible] binaural masking deteriorated signific[illegible] more in the vocally untrained and the [illegible] phonic persons than in singers, the dys[illegible] group showing the most rapid deterior[illegible] Further on, the fact that even without [illegible] the significantly smallest deviation fro[illegible] pitch of the reference tone was fo[illegible]

s, suggests that in persons with good function not only the kinesthetic but also ' ory control mechanism of phonation more efficiently than in other persons

on it should be mentioned that the pitch deviation of 0 21 semitones from e tones—as found in singers without pretty well corresponds to the re of Kerppola & Walle (1925) and is a smaller as measured by Sakurai & Oka- (1966), who in normal subjects reported pitch deviation of 2% (around 0 33

: significant widening of vibrato swing regular pitch fluctuations regularly seen the three groups has two causes first, of pitch modulation depends upon intensity Gemelli et al (1954), Sjostrom and Winckel (1974) have shown that the o swing becomes more pronounced at intensity levels, we recently demon that the irregular pitch fluctuations in a similar way (Schultz Coulon 1977) ll tested subjects increased the intensity of their singing voice as well as of their ng voice when being masked binaurally ly, a disturbance or interruption of stic automonitoring additionally influ tone stability, this has been pointed out eutsch & Clarkson (1959) and by Elliot & eller (1970), and it is supported by our observations that in some singers the o swing became irregular at maximum ing noise levels However in the indi l case it is impossible to decide which of two factors may be the more important the impairment of tone stability On the hand the fact that the increase in pitch lations upon masking remains relatively in all three groups suggests that tone lity depends to a greater extent upon the etic control mechanism than upon itory feedback In this regard we interpret remarkable *difference in tone stability* ween the dysphonic group and the normal jects (the average frequency range of pitch s of the dysphonic exceeded that of the normal group by about 0 5 semitones in all masking situations) as further evidence of an especially poor kinesthetic control ability in the dysphonic group

The phenomenon of "initial overswing" as described above can be explained in two ways either the sudden upward shift in fundamental frequency is accompanied by a short increase in the glottic air flow, causing the pitch eleva tion, or else the laryngeal muscles are initially adjusted to a higher activity level than the pitch of the new tone would require In any case, an initial overswing has to be regarded as incorrect pitch regulation which obviously mirrors the operational efficiency of the neuro muscular control system, because it occurs as frequently without as with interruption of auditory feedback (this is easily understood, since most of the time an initial overswing can not be perceived acoustically) In our experi- ment such inaccurate pitch regulation was seen more often in dysphonic patients than in the normal group, but rarely in singers This result again indicates that the neuromuscular control system works more efficiently in vo- cally trained people than in other ones

On the basis of these observations the fol lowing conclusions are drawn The ability of kinesthetic voice control appears to be closely related to the quality of vocal function The individual kinesthetic control ability can be evaluated by measuring the elevation of funda mental speech frequency and the accuracy of the singing voice during binaural masking as well as by observing the pitch fluctuations of a sustained tone and the precision of pitch control during a sudden upward shift in funda mental frequency Therefore the examination of kinesthetic control ability by means of fundamental frequency analysis seems to offer a possibility for obtaining objective informa tion about the quality of vocal function For the voice therapist such a possibility could be come of particular value not only for dia- gnostic purposes but also for an objective documentation of the terapeutic success of vocal training

ZUSAMMENFASSUNG

Die Arbeit berichtet uber Untersuchungen zur Frage ob eine Beziehung zwischen der kinasthetisch reflektorischen Phonationskontrolle und der Qualitat der Stimmfunktion existiert Bei 3 Personengruppen mit qualitativ unterschiedlicher Stimmfunktion (24 Sanger 32 stimmlich untrainierte Personen und 30 Patienten mit hyperfunktioneller Dysphonie) wurden Tonhohen und Laut [illegible]

[illegible] Lautstarke von 60 80 100 und 110 dB) mit Hilfe unseres kurzlich entwickelten Grundtonanalysators gemessen Das Experiment fuhrte zu folgenden Ergebnissen wahrend das Anwachsen der Sprechintensitat bei allen drei Gruppen ahnlich groß war unterschieden sie sich hinsichtlich der Anhebung der mittleren Sprechstimmlage signifikant die dysphonischen Patienten reagierten auf binaurale Vertaubung mit der großten Ton [illegible]

Sprechstimme Die Stimmgenauigkeit d h die Fahigkeit einen gegebenen Referenzton unter Vertaubungsbedingungen zu reproduzieren war bei Sangern weitaus großer als bei Normalpersonen und Dysphonikern Unabhangig von der Vertaubungssituation wiesen dysphonische Patienten eine deutlich geringere Stabilitat der Singstimme auf als die stimmgesunden Normalpersonen und zeigten auch am haufigsten eine unkorrekte [illegible]onhöhenregulation (ein sog initiales Uberschwingen) [illegible] schnellem Ubergehen von einem niedrigeren zu einem [illegible]ren Ton Vor dem Hintergrund der derzeitigen [illegible]siologischen Kenntnisse uber Tonhohen und Laut[illegible]rkekontrolle werden diese Beobachtungen als Nachweis dafür aufgefaßt daß die kinästhetisch reflektorische Phonationskontrolle bei trainierten Stimmen besser entwickelt ist als bei stimmlich untrainierten und bei dysphonischen Patienten woraus folgt daß eine enge Beziehung zwischen der Leistungsfähigkeit der kinasthetisch reflektorischen Stimmkontrolle und der Qualität der Stimmfunktion besteht Da diese Leistungsfahigkeit durch Beobachtung der Sing und Sprechtonhohenverände rungen unter Vertaubungsbedingungen gemessen werden kann erscheint eine objektive Bewertung der individuellen Stimmfunktion mittels der Grundtonanalyse moglich

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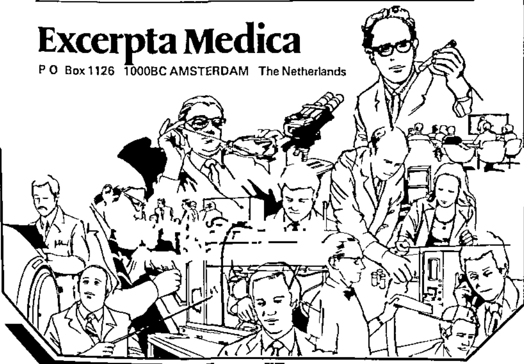
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PERIPHERAL VASOCONSTRICTION IN THE RAT IN RESPONSE TO SOUND

III *Dependence on Pause Characteristics in Continuous Noise*

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tract The peripheral vascular reaction to sound was ied in the non anaesthetized rat Arterial pulsations *e recorded by a non invasive technique from the tail* he animal The offset of a noise was found to be a k stimulus for vasoconstriction eliciting responses in y half of the presentations A vasoconstriction was ever regularly elicited by onset of sound after the of the pause The vasoconstriction was independent pause duration in the range 10 ms to 100 s For shorter ses the reaction was smaller the 2 ms pause elicited asoconstriction only occasionally It was especially ed that a 10 ms pause gave a larger response than a ms burst of noise The results were discussed in rela to decay of sensation and partial masking effects

e tail of the rat has suitable characteristics studies on the influence of sound on the rdiovascular system With a non invasive hnique arterial pulsations can be recorded non anaesthetized animals (Borg 1977) sound burst brings a drop in pulse ampli le whose duration rather than magnitude used *for a quantification of the response* nder these conditions the vascular response a noise burst follows an approximately ear intensity function down to the threshold hearing (Borg 1977) The sound energy is egrated over time in such a way that noise rsts with durations of 10 ms give significant smaller responses than stimuli of 0 1 or 1 s ration (Borg 1978*a*) During prolonged mulation the vasoconstriction slowly habit after approximately 1 hour of exposure 80 dB SPL noise the pulse amplitude is letely normal (Borg 1978*a*) It has also shown that the vasoconstriction is more sensitive to rapid changes than to slow changes in sound level (Borg 1978*b*)

The aim of the present study was to determine

(*a*) whether the acoustic vascular reflex is also sensitive to direction of change of sound level and

(*b*) whether it is dependent on the duration of a pause in a continuous noise in a way analogous to its dependence on the duration of a noise burst

METHODS

Two series of experiments were performed whereby two different ranges of pause duration were investigated 11 male adult rats participated in the first experiment and 10 in the second Two animals were experimentally naive and 8 animals took part in both sessions

Since a detailed presentation of the stimulus and recording system has been given earlier (Borg 1977) the following description of these will be brief During the experimental session the animal rested on a regulated heating pad in an individually adjustable net tube The bloodflow in the tail was assessed in terms of volume pulsations (Hellstrom 1975) recorded by a rubber balloon connected to a volume sensitive transducer (Elema 510C) In room temperature the tail artery was constricted and pulsations were minimal A moderate heating was necessary to induce vasodilatation The acoustic vascular reflex

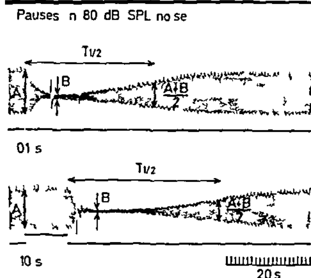


Fig 1 Record ngs of arterial pulsations obtained by a non nvas ve techn que from the tail of a non anaesthe t zed rat Decl ne of pulse ampl tude s el cited by pauses in 80 dB SPL prolonged noise *Upper record* 0 1 s pause *Lo er record* 10 s pause The vasoconstrict on is quant f ed by $T_{\frac{1}{2}}$ t me from end of pause unt l pulse ampl tude is halfway normal zed

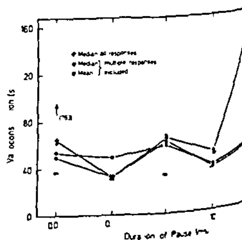
manifested itself as a decrease of pulse ampli de usually to less than 10% of the prestim value The vasoconstriction was quanti as the duration of the response from the art of the sound until the amplitude of the pulsation had returned halfway to prestimulus level ($T_{\frac{1}{2}}$ of Fig 1) The reasons for using the duration of the response rather than the change in pulse amplitude as a measure of the vaso constriction have been presented earlier (Borg 1977) A Lancing L75 loudspeaker placed 10 cm in front of the rat was used to deliver the sound stimuli

The animals were exposed for about 1 h to a continuous broad band noise (spectrum see Borg 1977) at a level of 80 dB SPL (sound pressure level re 20 μPa) The stimuli con sisted of pauses in the continuous noise (real ized as 65 dB attenuation) of vayring duration 10 ms 100 ms 1 s 10 s and 100 s in experi ment 1 2 ms 5 ms 10 ms and 100 ms in experiment 2 The rise and decay time of the noise breaks was 1 ms In the first experiment the stimuli were usually presented only 1–2 times in the second experiment they were pre sented 3–5 times with an interval of a k min

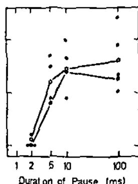
RESULTS

After approximately one hour s expos continuous broad band noise at 80 dB SP animal habituates with respect to pe vasoconstriction If the noise is ir erg however the vessel constricts aga n F shows two typical record ngs of art ral tion obtained with the non invasive tech from the surface of the tail of a non a tized rat The animal had been e several hours to continuous 80 dB SPL and the initial vasoconstriction had com habituated Typical observations d short 0 1 s and one long 10 s pause strated The gap in the ongo ng no s cated in the lower trace

It is seen that the pulse ampl tude is before the break fluctuating only in re respiration The short pause el c ts a constriction lasting for about 1 min Fro recording it is not possible to dec de the offset of the noise or the reiteration of noise after the interval is adequate s for the vascular reaction By us ng l pauses (10 s or 100 s) it is possible to d



F g 2 Durat on of vasocon tri tion as a f pause durat on Experiment 1 10 ms to 1 pause Med an and semi nterquart le range of all respo med an ±25 (●) and mean +S E M after mult ple vasocons rict ons ha e been e Measurements on 11 rats



? Durat on of vasoconstrict on as a funct on of on of pause in prolonged 80 dB SPL no se Experi ? 2 ms to 100 ms pauses Median ±25% (●) and +S E M (O) of all responses Measurements on 10

between the reactions to offset and onset noise In the lower recording in Fig 1 is seen to be elicited not alting the noise but by its onset after the However in about half of the record there was in fact a reaction to halting se This reaction was smaller variable y and usually of shorter duration than n to onset At the end of the pause of noise) a vasoconstriction was regu observed

s the offset of a sound is itself a weak lus for vasoconstriction in the rat under ent conditions

Fig 1 the reactions to 0 1 s and 10 s es were nearly identical The depend of vasoconstriction on the duration of se is more clearly illustrated in Fig 2 figure shows mean and median for the urements in experiment 1 pause length s to 100 s The vasoconstriction was ed as illustrated in Fig 1 by T_1 the from onset of noise until pulse had re half way to prestimulus value Since 25% of the pauses were not followed usual monophasic vasoconstriction (see 1) but by several consecutive constric two median values were calculated One an value was based on all responses (+) the other one did not include multiple (●) In addition mean value (O) standard error were calculated for the latter sample It is seen in Fig 2 that pauses between 10 ms and 10 s do not differ systema tically in their efficiency in causing vasocon striction If all the values are considered a 100 s pause seems to give a longer response than the short pauses This difference is not significant however (in only 6 out of 11 ani mals did the 100 s stimuli give greater re sponses than did the 10 s stimuli) It is interest ing to observe that the diminished efficiency in eliciting vasoconstriction so clearly seen for noise bursts with durations below 100 ms (Borg 1978*a*) is not seen for short pauses

Thus in the first experiment it was not possible to detect differences between short and long pauses In the second experiment still shorter pauses were investigated in the range from 2 ms to 100 ms Fig 3 shows average and standard error together with median and semi interquartile range for measurements in 10 rats Only few responses were multiple and were not excluded It is seen in Fig 3 that vasoconstrictions were usually smaller for 5 ms than for 10 ms pauses the difference is not significant ($p>0$ 05 Student s t test) The 2 ms pause (with a 1 ms rise and decay time) gave a significantly smaller vasoconstriction than the 10 ms pause ($p<0$ 001 Student s t test)

In conclusion the offset of a continuous noise may elicit a vasoconstriction but in most cases the vasoconstriction is caused by noise onset after the end of the pause A pause as short as 10 ms gives responses of the same length as pauses of many seconds dura tion

DISCUSSION

The present results show that the vasocon striction reflex is differentially sensitive to onset and offset of noise onset being a more efficient stimulus On the other hand the dura tion of the vasoconstriction is independent of the duration of the pause in the range 10 ms–100 s Only for pauses shorter than 10 ms does vasoconstriction decline

The present finding of the variability of

vasoconstriction on the offset of noise is in agreement with observations on heart rate response in rats made by Berg et al (1975) They found an acceleration of cardiac frequency on the halting of a tone, but only when a K-complex was elicited simultaneously in the electro encephalogram Generally speaking, heart rate did not alter on halting the tone Inglis (1974), on the other hand, found a significant average decrease in heart rate, both at onset and offset, of a 40 dB SPL noise Only one presentation of the stimulus was made for each animal in this latter work In the present study several pauses were presented and the animals were not experimentally naive Habituation to offset may explain the discrepancy between the present results and those of Inglis (1974) There is, however, no clear evidence for habituation within the individual experiments in the present study It seems rather to depend on the individual animal, some rats not showing responses to noise halt at all, and others doing so in a fairly consistent manner

The most interesting finding of the present dy is the very short integration time for reaction to a pause If a pause is to be étected, it is necessary that the neural activity elicited by the noise decline rapidly at the beginning of the pause In recordings from single fibres in the auditory nerve, it is evident that neural activity usually stops within a few ms after the end of a tone (Kiang, 1965) The spontaneous activity or the response to a subsequent test signal is, however, suppressed for 100 ms or more (Harris, 1977) The rate of decay of the auditory sensation has a similar time course A temporal gap between two noise bursts is detected when it is longer than about 3 ms (Plomp, 1964) but decay of sensation is not complete until after about 200 ms The present findings can be explained tentatively without assuming such differences between this reflex and perception

In order to tentatively explain the present findings, two concepts of psycho-acoustics can be considered namely decay of sensation (see e g Plomp, 1964) and partial masking (see e g Sharf, 1971) During the 200 ms
takes for the gradual decay of sens
the termination of a noise, an ensuing s
masked It was shown (Plomp 1964) t
sensational level of a 65 dB noise had d
to 45 dB 10 ms after noise termination
if the same noise is presented again
ms, it will exceed the sensation level
decaying sound by 20 dB On the othe
if a masked sound (Sharf 1971) is 20 dB
its masked threshold, it has a loudness
is almost identical with the loudness
same sound, unmasked Even though t
experiments are usually performed w
masking sound acting on another sou
difficult to see why the situation should
essentially the same for identical
Consequently, one has to expect that
will already reach its maximum effect
duration of 10 ms Shorter pauses ho
will be followed by smaller vasoconst
in the same way as the vasoconstric
dependent on the level of the noise
(Borg, 1977) Reservations must of co
made for differences between species
ferences between vasoconstriction a
ception, as well as for differences in the
of the stimuli used in the studies by
(1964) and Sharf (1971) and in the
work

If repeated vasoconstrictions in resp
sound do cause permanent changes
cardiovascular system, it can be sugg
the basis of the present experimen
continuous steady noise would be less
than an interrupted noise Further
steady noise with very short (e g
pauses would give stronger vasocon
than correspondingly short noise bursts
1978a) The present findings tally w
assumption that the temporal pattern
noise is of greater importance than the
itself for bringing about cardiovascular

ZUSAMMENFASSUNG

Die Reaktion auf Geräuschstimuli in periphe
gefäßen wurde an nichtanästhetisierten Ratten

der arteriellen Pulsationen im Schwanz mittels nicht invasiven Methode untersucht Es zeigte ein plotzlicher Abbruch des Gerausches (Fall ms) mit Hinblick auf die Gefäßkontraktion als ein Reiz auswirkt der nur in der Halfte der Falle ion auslost Durch Pausen in der Gerausch i wurden Gefäßkontraktionen ausgelöst die usenlangen von 10 ms bis 100 s unabhängig von der der Pausen waren Fur noch kurzere Pausen nahm tion ab und für 2 ms Pausen wurde nur gelegent ne Gefäßkontraktion ausgelost Besonders sei kt daß eine Pause von 10 ms eine großere Reaktion als ein Gerauschstoß von 10 ms

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THE ULTRASTRUCTURE OF THE BASILAR MEMBRANE IN THE CAT

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(Received December 29 1977)

Abstract A detailed study of the feline basilar membrane was performed in 13 cochleae with light microscopy and in six with electron microscopy The distribution of the mesothelial cells and homogeneous ground substance with the filaments was recorded and plotted as a function of length along the cochlear duct The width thickness and number of filaments were also measured In the lower basal turn the basilar membrane was narrowest and its entire thickness was occupied by filaments In the apical region the width was maximal and the filaments were fewer The density of the filaments counted in the bundles showed no significant difference along the cochlear duct or across the width of the basilar membrane but the number of filaments decreased markedly (approximately a ten fold difference) from base to apex The number of mesothelial cells increased towards the apex These [illegible]rphological characteristics may be related to the d[illegible]f[illegible]nt motion pattern of the basilar membrane along the [illegible] of the cochlear duct A discontinuity of the base[illegible]t membrane was noted in the apical region in all cochleae stud[illegible]ed These gaps seemed to provide structural evidence for the permeability of the basilar membrane in th[illegible]s area The vas spiral[illegible]s was present as a blood vessel in two specimens and only in the apical region Thus its function as the sole nutritional source for the organ of Corti is doubtful

The importance of the basilar membrane in the frequency selectivity of the cochlea has been recognized for more than a century (Helmholtz 1863 Hurst 1895 Zwislocki 1953 von Bekésy, 1960 Tonndorf 1960 Dallos 1970 Rhode, 1971, Wilson & Johnstone 1975) It is clear that different sound stimuli produce a maximum deflection of the basilar membrane at different levels of the cochlea and that the motion pattern of the basilar membrane is determined by its morphological features These factors suggest that there are some structural differences in the basilar membrane along the length of the cochlear duct

Other previous studies of the basil[illegible] membrane morphology (Retzius 1884 Eng[illegible] 1955, Iurato 1962 1967, Angelborg & [illegible]strom 1974 Shinozaki & Miyoshi 19[illegible] been reported In this study a detailed [illegible]tion of the different elements that comp[illegible] basilar membrane is presented w[illegible] phasis on their distribution across the [illegible] Corti and along the length of the coch[illegible] The width thickness and number of fil[illegible] were measured The cat was chosen as [illegible]perimental animal since most of the av[illegible] physiological and anatomical data are [illegible] this animal

MATERIALS AND METHODS

A total of 19 cat cochleae were studied [illegible] light microscopy and 6 by electron [illegible]scopy

Eight temporal bones from 4 adu[illegible] weighing 2 to 3 5 kg were fixed in Heide[illegible] Susa, embedded in celloidin (Sch[illegible] 1968), and serially sectioned in the hor[illegible] plane at 20 μm thickness Every ten[illegible] was examined under the light microscop[illegible] the cochleae were reconstructed (Sch[illegible] 1953) The average length of the basilar [illegible]brane was found to be 23 6 mm±0 9[illegible]

[1] [illegible] 02114 USA

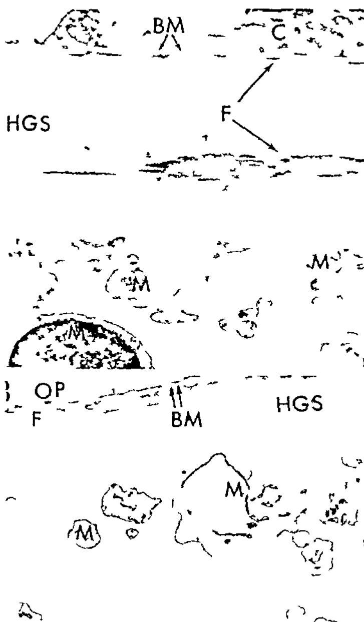
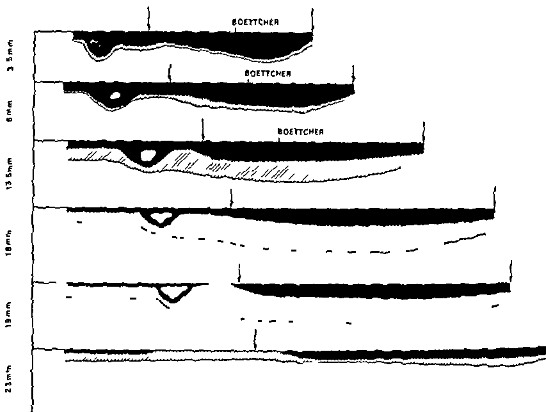


Fig 1 Electron micrographs showing the different elements that comprise the basilar membrane *BM* basement membrane *F* filaments (upper and lower layers) *M* mesothelial cells (A) Pars pectinata at the level of the Claudius cells (*C*) in the apical region ×8000 (B) Pars tecta at the level of the outer pillar cell (*OP*) in the second turn Note the different distribution of the filaments (*F*) they form a single layer at this level ×6750

One ear from each of 5 adult cats was embedded in Epon and studied by surface preparation The width of the basilar membrane was determined from the nucleus of the inner pillar cell to the basilar crest (junction of the basilar membrane and spiral ligament) To define the exact limits radial 2 μm thick sections were also made

One ear from each of 6 cats was used for electron microscopic study To allow direct comparison between young and adult the animals were divided into two groups one a group of 3 small animals (1 09 to 1 27 kg) and the second a group of 3 adult animals (3 09 to 3 4 kg)

The cochleae were intravitally perfused through the opened oval and round windows with Karnovsky s solution (1965) at pH 7 3 The cochleae were postfixed in 1% phosphate buffered osmium tetroxide and embedded in



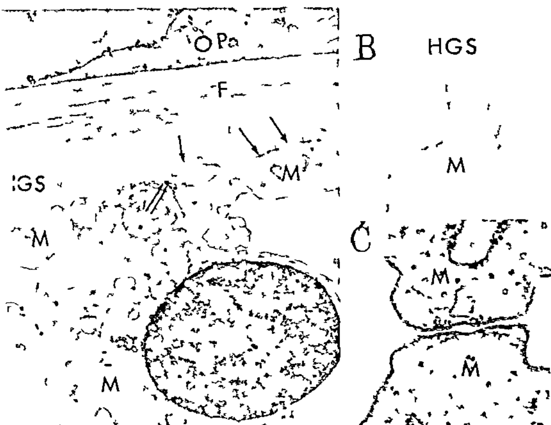
2 Schematic drawing showing the variation in thick of the basilar membrane along the cochlear duct at erent points in the same cat The black and shaded ions represent the outline of the homogeneous ground stance with the filaments included and the area occu pied by the mesothelial cells respectively Th axis corresponds to the distance from the base each case the arrow on the right side indicat crest and the left arrow indicates the demarc tween the pars tecta and the pars pectinata

Epon (Kimura, 1967) Each embedded cochlea was then bisected in a plane perpendicular to the 5 mm point of a ruler placed against the round window and promontory In all cases, two pieces were obtained from each turn corresponding to the following locations lower basal turn, upper basal turn, lower second turn, and two from the apex The location (±1 mm) of each piece along the length of the cochlear duct was determined and plotted on a standardized cochleagram In all specimens radial sections were made in a plane parallel to the long axis of the outer hair cells of the organ of Corti using an LKB Ultrotome

In two cochleae one additional piece was taken from each turn and was serially sectioned in a transverse plane to the basilar membrane, from the basilar crest of the spiral ligament to the habenula perforata The sections were stained with uranyl acetate citrate Photographs were taken with mens Elmiskop I at magnifications from 1 000 to 40 000×

Measurements of the thickness of the membrane were made from the ba membrane to the tympanic surface of mogeneous ground substance and from points within the mesothelial cells al which seemed to best define the genera of this area Along the width of the membrane, an average of ten differen for each section was used to make the struction

The density of filaments in the b ($F/\mu m^2$) was determined The average of filaments per micron length (F/μ obtained by counting the total number ments and relating that number to the le



! (A) Rad al section of the bas lar membrane at the of the outer p llar cell (*OP*) Occas onally hem osomes (s ngle arrows) are seen at the sites of con between mesothel al cells (*M*) and the homo- us ground substance (*HGS*) The double arrows the cellular junct on between two mesothel al cells l ke) *F* f laments ×11200 (B) H gher magnif cat on electron micrograph of the hemidesmosome between the homogeneous ground substance (*HGS*) and the mesothel al cell (*M*) ×66000 (C) Electron micro- graph show ng a desmosome l ke junction between two mesothelial cells (*M*) Note that the intercellular space is smaller at the s te of the junct on ×77000

asilar membrane contained within it The of filaments was defined by using a com ating polar planimeter

FINDINGS

Ultrastructure of the Basilar Membrane

basilar membrane underlines the organ of ı from the tympanic lip of the limbus spi to the basilar crest of the spiral ligament sement membrane forms a line of de kation between the cells of the organ of ı and the basilar membrane Three main can be distinguished a fibrous por a homogeneous ground substance and the mesothelial cells (Fig 1) The basilar mem brane is divided into two zones the pars tecta which extends from the tympanic lip of the limbus spiralis to the outer pillar cell and the pars pectinata which extends from the outer pillar cell to the basilar crest of the spiral liga ment

The *homogeneous ground substance* has an amorphous and filamentous appearance It lies between the basement membrane and the mesothelial cells The fibrous portion is lo cated within it The distribution of the homo- geneous ground substance (Fig 2) varied not only along the length of the cochlear duct but also across the width of the basilar membrane

Table II

	Filaments per μm²			*Filaments per μm length*		
	Apex (23 mm)	Second (17 mm)	Basal (9 mm)	Apex (23 mm)	Second (17 mm)	Basal (9 mm)
Claudius						
Upper	1 580	1 664	1 400	137	692	777
Lower	1 440	1 460	1 450	343	655	958
Hensen						
Upper	1 358	1 479	1 504	112	527	656
Lower	1 945	1 548	1 400	364	876	935
Deiters						
Upper	1 622	1 231	1 592	74	453	602
Lower	1 441	1 359	1 375	402	949	1 089
Outer pillar	1 397	1 587	1 720	487	1 579	1 840
Tunnel	1 380	1 764	1 552	512	1 131	1 739
Inner pillar	900	1 200	1 269			

length of the cochlear duct (Fig 2) There was an increase from base to apex and a decrease at the apical end of the cochlea The maximum thickness was found in the upper second or in the lower apical turns In the lower basal turn ... was only one very thin layer (less than ...m thick) of cell nuclei and prolongations ... each other and lying closely adja... to the homogeneous ground substance This appearance changed toward the apex and in the upper second turn three or more nuclear layers were present Also the number of cell processes increased greatly with many free spaces between them

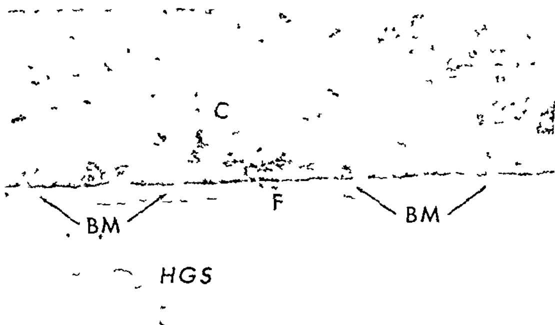
The distribution of mesothelial cells across the width of the basilar membrane was very similar in both the pars tecta and the pars pectinata and it followed the pattern described above The only differences were that in the pars tecta there were usually fewer cells and the area occupied by them was thinner than in the pars pectinata

The total number of cells located across the width of the basilar membrane and the mean fraction of cells per micron width were determined by counting the nuclei in each radial section There was an increase of mesothelial cells from base to apex except at the apical end of the cochlea where they decreased again These values contrast with those found by Angelborg & Engstrom (1974) in the g... pig where the number of cells was ... greater in the basal turn

The *fibrous portion* was composed of r... ally oriented filaments with an uneven ... profile (Fig 4B) The diameter of thes... ments ranged from 90 to 120 Å Each fil... was rectangular or diamond shaped in c... section with four or five subfibrils (Fig 4... Other investigators have found fibr... similar morphological characteristics ... vestibular perilymphatic space of rats (Ka... ton 1967) the spiral ligament of the R... monkey (Takahashi & Kimura 1970) and basilar membrane of the guinea pig (K... 1975)

The distribution of filaments along the ... of the basilar membrane differed in the ... tecta and the pars pectinata In the pars ... they lay side by side within the homo... ground substance forming a cont... compact layer while in the pars pectina... were grouped in bundles and were arra... two or more strata Homogeneous grou... stance was present between the bundles ... the two strata

The density of the filaments in the b...



6 Electron micrograph of the bas lar membrane at level of the Claud us cells (C) in the apical region arrows show the locat on of the basement membrane (BM) HGS homogeneous ground substance F fila ments ×19000

' the number of filaments per micron length m two cats are shown in Tables I and II density of filaments ($F/\mu m^2$) in the showed no significant difference along length of the cochlear duct (basal second apical turns) or across the width of the ılar membrane (1 430±250) However there s a decrease in density at the level of the er pillar cell mainly in the apical region and he second turn In the tunnel of Corti some filaments appeared to change their radial and assumed an oblique orientation ᵃrefore underneath the inner pillar cell the ments were scattered and they appeared to embedded in dense conglomerates of homo- ᵒus ground substance

the number of filaments were con in relation to the length of the basilar significant differences were ob- ᵛd not only across the width of the mem ne but also along the cochlear duct Across width of the basilar membrane these differences were more evident in the pars pec tinata In the upper layer the filament popula tion decreased progressively from the basilar crest to the outer pillar cell while in the lower layer the opposite occurred (Tables I and II) This observation was in accord with the fact that the bundles in the upper layer became thinner A discontinuity in the upper bundles was present (in both second and apical turns) at the level of the Hensen cell area The lower layer on the other hand contained larger bundles These complementary changes in the two strata arose from the presence of inter mediate bundles which seemed to descend from the upper layer to the lower layer In the lower basal turn it was not possible to obtain separate data for each strata because there were so many intermediate fibers that no dis crete layers could be distinguished

At the level of the Deiters cells both layers were very close and they joined underneath the outer pillar cell to form the pars tecta At

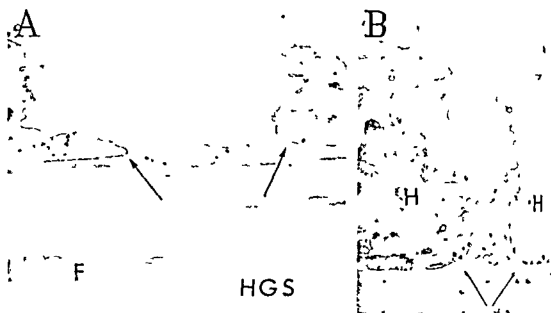


Fig 7 Electron micrographs showing the discontinuity of the basement membrane (*arrows*) at the level of the Hensen cells (A) Radial section in the 21 mm area *F* filaments *HGS* homogeneous ground subst[illegible] (B) Cross section in the 22 mm area *H* [illegible] ×13 700

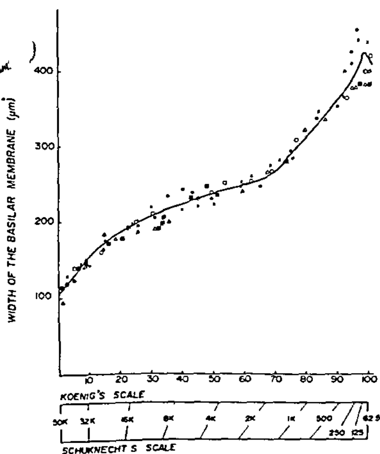


Fig 8 Graph showing the [illegible] the feline basilar membrane The [illegible] µm is plotted as a function of [illegible] centage of the cochlear length The [illegible] and Schuknecht frequency [illegible] presented along the bo[illegible]

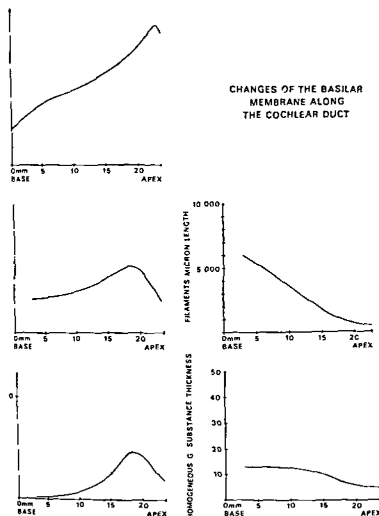


Fig 9 Composite diagram showing the changes of the basilar membrane and the elements that comprise it along the length of the cochlear duct The graphs showing mesothelial cell homogeneous ground substance and total thicknesses were obtained at the level of the pars pectinata

evel of Corti's tunnel a group of filaments ched from this filamentous compact layer l an arc around the vas spiralis area, which they again joined the filaments of tecta The number of filaments forming is arc was variable, that is they were numerous in the basal turn and very in the apical region The filaments per n length were not determined at the level inner pillar cell because they were so dispersed that it was impossible to ine the area containing filaments

number of filaments per micron length varied greatly along the cochlear duct In ower basal turn (4 mm area) the entire s of the basilar membrane contained filaments (5400±700) The number decreased toward the apex, 1340±70 in the upper second turn and 570±110 in the apical end It represents approximately a ten fold difference between base and apex Therefore, the thickness of the area containing filaments decreased markedly along the length of the cochlear duct (Fig 5)

The *basement membrane* is a semi opaque linear structure of finely granular appearance which forms a boundary between the organ of Corti and the basilar membrane (Fig 6) It was separated from the supporting cells of the organ of Corti by a gap that varied between 200 and 400 Å This space was randomly occupied by a granular material which seemed to have a

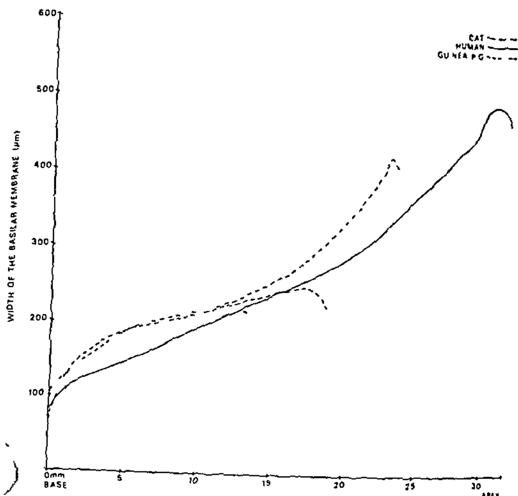


Fig 10 Comparative graph of the variation in basilar membrane width for three different species Human data were taken from Wever (1938 1949) and guinea pig data from Fernandez (1952) The vertical axis corresponds to the width of the basilar membrane in µm ... zontal to the length of the cochlear duct in ... the human 18 mm for the guinea pig and 21 ... cat)

density comparable to the basement membrane itself The thickness of the basement membrane ranged from 400 to 600 Å No differences were found between the pars tecta and the pars pectinata

In the upper second turn and in the apical region the basement membrane was discontinuous (Fig 7) It folded over two adjacent Hensen cells and surrounded them, ending at the cell membrane and leaving a communication between the intercellular spaces of the organ of Corti and the basilar membrane Also at this level the upper layer of filaments was very thin and a discontinuity between the bundles was observed Such gaps were present after the 18 mm area in all the cochleae studied, and in two cases they were even observ... lower second turn They varied grea... with a range of 0 09 to 1 8 µm The... combined with the loose distribut... mesothelial cells, would allow free ... the perilymph with the intercellular s... the organ of Corti

II *The Width of the Basilar Mem...*

Measurements of the width of the ... membrane are shown in Fig 8 The... progressive increase from base to ap... the maximum width was noted in ... Other than individual variations in ... curves, there were no significant d... that could be related to the age of the ...

e curve representing the average width of)asilar membrane showed a gradual in- ,e from the round window to the lower nd turn (65% of the cochlear length) At level a sharp change in the slope of the e was observed This change occurred e area between 1 and 2 kHz in the Koenig ?) and Schuknecht (1974) scales This)er increase in the width of the basilar ibrane was maintained until the maximum reached A sudden decrease in width was rved in the last 1 mm

ıe maximum mean width of the basilar ıbrane was 426 μm and the minimum was μm, which represents an approximately fold increase from base to apex

II *Thickness of the Basilar Membrane*

total thickness of the basilar membrane ended on the distribution of the homo- ,ous ground substance with the filaments the mesothelial cells (Fig 2) It should be illed that they varied conversely along the hlea, thus, as the thickness of the homo- cous ground substance and filaments de- ısed from base to apex, the area cor-)onding to the mesothelial cells increased he total thickness of the basilar membrane wed a progressive increase from the basal ı to the apical region, essentially due to an rease in the population of mesothelial cells ˀ maximum thickness was found at the el of the upper second or in the lower apical ıs At the apical end of the cochlea the total kness decreased again

DISCUSSION

e importance of the basilar membrane in the chanical behavior of the cochlear partition › been recognized for more than a century lmholtz (1863) formulated the resonance ory, suggesting that every tone has a spe locus along the basilar membrane where fibers would act as resonators Hurst on the other hand, suggested the trav- wave theory and considered that the wave of displacement progressed systemati cally along the basilar membrane producing a local stimulation in its path Von Bekesy (1960) made direct measurements of the vibratory characteristics of the cochlear partition He showed that the sound stimulus produced a traveling wave and that each frequency maxi- mally excited a different part of the cochlea Furthermore, the maximum deflection of the cochlear partition produced by high fre quencies occurred in the basal portion, where as that produced by low frequencies occurred in the apical region Wever et al (1954) and Tonndorf (1959) established that traveling waves were produced by the transfer of energy from one perilymphatic scala to the other across the partition However, the vibratory pattern was determined by the morphological properties of the membranous partition itself and would also be determined by the proper- ties of the basilar membrane Therefore, if the mechanical behavior of the cochlear partition varied from base to apex, this might be cor- related with some morphological changes in the basilar membrane along the cochlear duct (Fig 9) Such changes would determine the relative stiffness or elasticity of the basilar membrane at different levels

After von Bekesy's experiments, it was known that the basilar membrane is not under lateral tension, but it has some stiffness or resistance to displacement, which is one hundred times greater in the base than in the apex in the human (von Bekesy, 1960) This difference can be explained, at least partially, by the differences in width, thickness and number of filaments along the length of the cochlear duct Many previous studies have been concerned with the width of the basilar membrane (Hensen, 1863, Guild 1927 Wever, 1938, Perlin, 1946, Fernandez, 1952 Igarashi et al , 1968, Antoli Candela et al , 1976) A progressive increase from base to apex was always reported In this study the width of the basilar membrane was also meas ured and the results were similar (Fig 8) The narrowest width (106 μm) was located in the

basal region and the maximum (406 μm) in the apex (approximately a four fold difference) The increase in width of the basilar membrane was not gradually progressive A sharp change in the slope of the curve was observed at about 15 mm from the basal end of the cochlea

Although lateral tension has not been demonstrated in the basilar membrane, it is probable that an important contribution to the resistance to displacement of the membrane could be provided by the radial filaments The density of the filaments within the bundles remained the same along the length of the cochlear duct, but the number of filaments which occupied the thickness of the basilar membrane underwent a great reduction from base to apex At the lower basal turn, they numbered roughly 6000/μm which contrasted with the 600/μm in the apical region (approximately a ten fold difference)

The thickness of the basilar membrane (Fig 2) increased from base to apex with a maximum at the level of the upper second turn or e lower apical turn This change in thickness correlated with the increased number of esothelial cells towards the apex Unfortunately, the role of the mesothelial cells in relation to the stiffness of the basilar mem brane is not known A further point of interest is the loose distribution of the mesothelial cells in the apical region At this level they comprised the main element in terms of thickness It is possible that in this apparently weaker portion of the basilar membrane where the homogeneous ground substance was very thin or absent in some areas and the filaments were fewer, they provide some kind of flexible sup port that acts as a cushion or energy absorber for strong vibrations

Curves showing the mean widths of the basilar membrane for the cat guinea pig and human appear in Fig 10 The human data were obtained from Wever (1938 1949) and the guinea pig data from Fernandez (1952) The shapes of these curves are similar Each curve shows an increase in width from base to apex, however, each has its own characteristic rate of interest The minimum values f[illegible] species are more similar than the [illegible] At the basal end the values are 80 μ[illegible] human, 106 μm for the cat, and 70 μ[illegible] guinea pig At about a half turn from [illegible] end where the maximum width is [illegible] values are 498 μm for the human 4[illegible] the cat, and 250 μm for the guinea pi[illegible] tion to cochlear length the com[illegible] mean values are 32 mm distributed [illegible] for the human, 23 66 mm in 3½ tur[illegible] cat, and 18 8 mm in 4 turns for the g[illegible] The maximum width is noted in [illegible] region (Fig 10) where the maxim[illegible] tion of the basilar membrane corres[illegible] the low frequencies The limit for the [illegible] cal frequency analysis at this level is [illegible] the human and 200 cps in the guinea [illegible] Békesy, 1960) In the cat this li[illegible] sidered to be 62 5 cps using behav[illegible] (Schuknecht, 1953) It should be n[illegible] these limits of the auditory field (h[illegible] guinea pig) are defined in terms of m[illegible] *frequency analysis and further val[illegible]* obtained through neural processes

Fleischer (1976) suggested that t[illegible] mum amplitude of displacement fo[illegible] frequency occurs at the same wid[illegible] basilar membrane in all mammals T[illegible] improbable since the maximum wid[illegible] basilar membrane for the guinea pig [illegible] which corresponds to the 14 mm area [illegible] basilar membrane At this area the c[illegible] quency is about *1500 cps on the S[illegible]* scale If Fleischer's theory were c[illegible] would imply that the guinea pig ha[illegible] chanical response to frequencies be[illegible] cps, and yet the accepted lower freq[illegible] 200 cps Therefore, other charact[illegible] the basilar membrane such as th[illegible] number of filaments must also have [illegible] fluence on mechanical frequency an[illegible]

Very few studies on the thickne[illegible] basilar membrane in *different spec[illegible]* been carried out Fernandez (195[illegible]) that the thickness of the homogene[illegible] substance in the guinea pig was 7 4 μ[illegible]

nning of the first turn and 1 34 μm at the al region but he did not mention at which l across the width of the basilar membrane measurements were obtained In the ent study an average of ten points for y section was used to make a graphic re struction The maximum value for the ogeneous ground substance in the pars inata in the lower basal turn was roughly o 14 μm and this value decreased to 5 μm e apical region If the total thickness were sidered, the corresponding values would 14 to 15 μm and 26 μm (upper second) Unfortunately, no data related to human lar membrane thickness could be found

n all specimens studied, a discontinuity in basement membrane between the Hensen was found in the apical region (usually ond the 18 mm area) The possibility of matic artifact due to drilling of the apex overruled by the finding of these gaps in cochlea in which there was no drilling se gaps provide structural evidence of the communication between the intercellular ces in the organ of Corti and the scala tym i through the basilar membrane, as previ ly described by Altmann & Waltner (1950) ındorf et al (1962), Nomura (1968), von Il & Vosteen (1969), Masuda et al (1971), Lorenzo et al (1972) and Duvall & Suther d (1972) At this level in the pars pectinata upper layer of filaments was very thin and iscontinuity between the bundles was ob ved The loose distribution of the meso-lial cells would allow free contact between homogeneous ground substance and peri iph Thus, with no barrier in the basilar mbrane, such gaps could permit free pas-e of perilymph between the intercellular ces of the organ of Corti and the scala tym ii

The vas spiralis, located beneath the tunnel Corti was first described by Retzius (1883) the rabbit embryo and in the fetal cat Smith 954) performed a vascular study in 8 cats, d in 6 of them the vas spiralis did not present a blood vessel It was therefore concluded that the vas spiralis had little, if any, signifi cance in itself Lawrence (1966) reported that the interruption of blood flow through the vas spiralis in the guinea pig caused a loss of hair cells and suggested that it was an important source of nutrition for the organ of Corti Kikuchi & Hilding (1967) considered that the vas spiralis in Shaker-1 mice underwent involution and lost its lumen 2 weeks after birth which could be an important factor in the subsequent degeneration of the outer hair cells

In this study the vas spiralis was observed in only two of the six specimens studied, and in both it was located in the apical region Re lating this observation with Retzius' findings (1884) which showed that this vessel disappeared in the apex in the 30-day fetal cat though it was present in all coils at an earlier stage, it seems clear that the vas spiralis un dergoes involution during the cat fetal period Thus, when the vas spiralis remains in the adult animal, it could probably be considered an embryo-vestige No correlation with the age of the animals (young or adult) was noted, nor was there any obvious pathology at the level of the organ of Corti Therefore, the function of the vas spiralis as the sole nutri tional source for the organ of Corti is doubtful

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ZUSAMMENFASSUNG

In 6 elektronenmikroskopischen und 13 lichtmikroskopischen Cochlea Praparaten wurde eine eingehende histologische Untersuchung der Membrana basilar vor genommen Die Verteilung von Mesothelıumzellen und der von Filamenten durchsetzten Grundsubstanz wurde als Funktion zur Langendimension des Ductus cochlearis dargestellt Die Weite Dicke und Zahl der Filamente wurde ausgemessen In der untern Basalwindung wo die Basilarmembran am schmalsten ist ist sie völlig mit Fila menten durchsetzt In der Spitzenwindung wo die Basi

larmembran am breitesten ist sind die Filamente weniger zahlreich In der Bündeldichte der Filamente wurden keine signifikanten Unterschiede langs des Ductus coch learis festgestellt jedoch nahm die Zahl der Filamente von der Basalwindung gegen die Spitzenwindung etwa um das Zehnfache ab Andererseits nahm die Zahl der Mesotheliumzellen gegen die Spitzenwindung hin zu Diese morphologischen Unterschiede korrelieren wahrschein lich funktionell mit einem differenten Bewegungsmuster der Basilarmembran In allen untersuchten Spezimen wurden in der Apikalwindung Diskontinuitaten in der Basilarmembran gefunden Diese Lucken weisen auf spezifische Permeabilitatseigenschaften der Basilar membran in der apikalen Region hin Das Vas spiralis konnte als Blutgefaß nur in 2 Spezimen positif identifiziert werden und zwar nur in der Spitzenwindung daher scheint seine Funktion als alleinige Ernährungsquelle fur das Cortische Organ zweifelhaft

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ION TRANSPORT IN THE COCHLEA OF GUINEA PIG

II Chloride Transport

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Abstract The Cl transport across the endolymph–perilymph barrier in guinea pigs was studied by perfusing the perilymphatic space with artificial perilymph containing ^{36}Cl and measuring the uptake of ^{36}Cl in the endolymph In normal animals no marked difference in Cl concentration was found between the endolymph and perilymph The data showed that the uptake of ^{36}Cl in the endolymph could be represented by a simple exponential function of the perfusion time the rate constant being 0 01 min^{-1} The concentration of ^{36}Cl in the endolymph was greater with perfusion of the scala vestibuli than with perfusion of the scala tympani indicating that Reissner s membrane is more permeable to Cl than the rest of the endolymph–perilymph barrier Anoxia and local application of ouabain decreased the Cl concentration and ^{36}Cl uptake in the dolymph Our results imply that the endocochlear po- al is the principal driving force for unidirectional flux Cl from perilymph to endolymph

The chloride concentration in the cochlear fluids has been studied by several investigators (in cats, Ledoux, 1950, Citron et al , 1956, in guinea pigs, Citron et al , 1956, Rauch, 1964, Smith et al 1954, in rats, Bosher & Warren, 1971) These studies indicate that the concentration of $^{-}$Cl in the endolymph ranges from 160 to 110 mEq/l and that there is no marked difference in $^{-}$Cl concentration between the endolymph, perilymph or cerebrospinal fluid Recently Bosher et al (1973) found decreased $^{-}$Cl concentration in the endolymph in rats treated with ethacrynic acid and concluded that the observed change in Cl concentration must arise from the direct action of ethacrynic acid on the cochlea

As the endolymph is positively polarized in the resting state, $^{-}$Cl will tend to leak into the endolymph Nevertheless the fact that the actual concentrations of $^{-}$Cl in the end[...] are almost equal suggests the exis[...] $^{-}$Cl pump which extrudes $^{-}$Cl from the [...]lymph, as has been postulated by J[...] (1967) However, extensive studies o[...] transport across the endolymph–pe[...] barrier have not been available It is th[...] worthwhile investigating the $^{-}$Cl m[...] across the endolymph–perilymph bar[...] utilizing the radiotracer technique a[...] tempting to examine the potential alter[...] the $^{-}$Cl transport mechanism caused by [...]ous ototoxic agents

In the work reported here we stud[...] transport across the endolymph–per[...] barrier under the condition of continu[...] fusion of the perilymphatic space in the [...]lea of guinea pig with artificial perilym[...] taining ^{36}Cl Our data suggests that in th[...]ing state the $^{-}$Cl concentration in the [...]lymph is maintained by an outward f[...] is energy-dependent and a passive ma[...] which is driven by the endocochlear p[...] (EP)

METHODS

Healthy guinea pigs (NIH strain 300 [...] anesthetized with pentobarbital sod[...] used throughout our experiments The [...]ods used for the recording of the co[...]tentials, perfusion of the perilympha[...] and collection of the endolymph and [...]lymph were the same as those described [...] preceding paper (Konishi et al , 197[...]

le I −Cl *concentrations in cochlear fluids and EP*[a]

	Perfusate (mM)	Perilymph (mM)		Endolymph (mM)	EP (mV)	n
		Scala vestibuli	Scala tympani			
perfused cochlea		127 8±5 3	128 6±5 1	131 0±5 6	81 3±2 0	7
ised cochlea	152 2±12 8	138 8±4 7	139 2±4 9	138 9±5 8	83 2±2 2	5

lues are means±standard deviations n=number of guinea pigs The scala vestibuli and tympani were perfused period of 60 min Samples were taken at the end of perfusion

lifficult to collect more than one sample of endolymph and perilymph in the scala tibuli and tympani, separate animals were d for each experiment An exception to the ve was made in one set of experiments, which the radioactivity in the perilymph was ermined as a function of time in the same mal

⁶Cl (3 07×10⁵ year half life) was obtained as ieous NaCl solution from Amersham Sear- Illinois The original solution was diluted th modified Ringer's solution which had the lowing composition (mM), NaCl 106, KCl 5, Cl_2 2, NaH_2PO_4 1, MgCl 1, $NaHCO_3$ 12, glu- se 11 The −Cl concentration in the per- sate ranged from 160 to 140 mM The activ- of ^{36}Cl in the perfusate was approximately μCi/ml The perfusate was kept at room tem rature (24°C) The activities of ^{36}Cl in per- sate, perilymph and endolymph were deter- ned using a liquid scintillation counter A unting time of 20 minutes was sufficient to duce standard deviation in samples from unting errors to less than 1% of total counts

To determine the −Cl concentration in the rfusate, perilymph and endolymph, a modi cation of an electrometric technique devel ped by Ramsay, et al (1955) was employed he Cl was titrated with the silver ions re- ased by passing a constant current of 100 nA rough a silver wire The current to liberate ie silver ions was generated by a solid state onstant current injector (Chen and Konishi, preparation) The titration time defined by he elapsed time between the onset of current pplication and end point, is linearly propor ional to the −Cl concentration The sample volume was kept constant (approximately *10* nl) The standard deviation was less than 1% when replicate samples of 100 mM chloride solution were titrated

RESULTS

Perfusion of Scala Vestibuli and Tympani

1 *Normal guinea pig*

The changes in the cochlear responses to sound stimuli observed during perfusion of the perilymphatic space with artificial perilymph containing ^{36}Cl were similar with those ob- served during perfusion with ^{42}K and ^{22}Na which were described in our preceding paper (Konishi et al , 1978) To summarize briefly, the cochlear microphonics (CM) were only slightly suppressed in a short period of perfu- sion but gradually decreased with perfusion times longer than 20 minutes (<30% of the in itial CM) The action potential of the auditory nerve (AP) and the summating potential (SP) showed temporary changes after the perfusion was initiated but remained stable during the remaining perfusion period The mean EP re- corded at termination of perfusion with vary- ing durations (10 to 90 minutes) was 82 7±3 8 mV (n=21) which was comparable to 81 3± 2 0 mV (n=7) recorded from the non per- fused cochlea It was noted that the temporal changes in the cochlear potentials were not affected by variation of the −Cl concentration in the perfusate ranging from 160 to 140 mM

The mean −Cl concentrations in the peri lymph of the scala vestibuli and tympani were 127 8±5 3 mM and 128 6±5 1 mM respec- tively in seven non perfused cochleae (Table

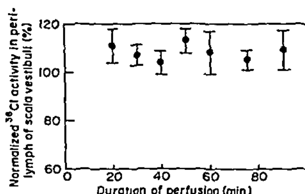


Fig 1 Mean concentrations of ^{36}Cl in the perilymph of the scala vestibuli for various durations of perfusion The perfusate was introduced into the scala tympani of the basal turn and both the scala vestibuli and tympani were perfused The ^{36}Cl concentrations in the scala vestibuli are normalized by taking those in perilymph of the scala tympani as 100% Vertical range bars indicate standard deviations Four guinea pigs were used for each perfusion time

1) The mean $^{-}$Cl concentration in the endolymph was 131 0±5 6 mM No significant differences in the $^{-}$Cl concentration were found between the endolymph and perilymph When the perilymphatic space was perfused for 60 minutes in 5 animals, the mean $^{-}$Cl concentration in the perilymph of the scala vestibuli and tympani was 138 8±4 7 mM and 139 2±4 9 mM respectively The mean $^{-}$Cl concentration in the endolymph was 138 9±5 8 mM and the mean EP was 83 2±2 2 mV Significant differences were observed in the $^{-}$Cl concentration when the data from the perfused cochlea were compared with those obtained from the non perfused cochlea (endolymph to endolymph and perilymph to perilymph, *t* and *u*-tests $p=0$ 05) However, *u*- and *t* tests did not reveal any significant differences ($p=0$ 05) in the $^{-}$Cl concentration between the endolymph and perilymph in either perfused or non perfused cochleae

When the perfusate containing ^{36}Cl was introduced into the scala tympani and both scala vestibuli and tympani were perfused for a period ranging from 10 to 90 minutes the concentration of ^{36}Cl in the perilymph of the scala vestibuli increased rapidly and reached the level of ^{36}Cl concentration in the perilymph of the scala tympani 10 minutes after perfusion was initiated The mean ^{36}Cl concentration in samples taken from the scala vestibuli remained higher than the ^{36}Cl concentration in the perilymph of the scala tympani as perfusion was prolonged as shown in Fig 1

The ^{36}Cl concentration in the perilymph was found to decrease rapidly in 3 animals after the perfusion was discontinued The clearance was much faster in the perilymph of the scala tympani than in the perilymph of the scala vestibuli (Fig 2) The rate of decrease of ^{36}Cl was essentially the same as that of and ^{22}Na which was reported in the previous paper (Konishi et al , 1978)

As shown in Fig 3 the presence of [illegible] the endolymph could be detected 10 [illegible] after the perilymphatic space was perfused with ^{36}Cl solution The concentration in the endolymph increased as the duration of perfusion was prolonged but it did not [illegible] ^{36}Cl concentration in the perilymph [illegible] minutes of perfusion, the ^{36}Cl concentration in the endolymph had reached about 60% [illegible] ^{36}Cl concentration in the perilymph of the scala tympani

In the steady state the net flux of Cl [illegible] the endolymph–perilymph barrier is [illegible] concentration of ^{36}Cl in the endolymph

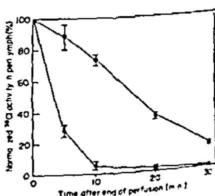
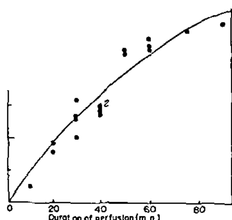


Fig 2 Clearance of ^{36}Cl after the end of perfusion [illegible] perilymphatic space ● ^{36}Cl concentration in perilymph of the scala vestibuli [illegible] ^{36}Cl concentration in perilymph of scala tympani The ^{36}Cl concentrations are normalized by taking those obtained at the end of perfusion as 100% Three guinea pigs were used [illegible] vertical range bars indicate the standard deviation



3 Uptake of ^{36}Cl in the endolymph as a function of sion duration Both scala vestibuli and tympani were sed with ^{36}Cl solution The ^{36}Cl concentration in the ymph is normalized by taking those in the perilymph scala tympani as 100% ● Data points The line is ast square best fit to the points (see text) Numeral 2 ates two overlapping data points

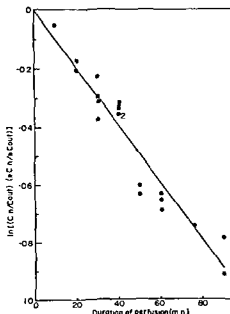


Fig 4 $\ln\{(C_{in}/C_{out})-(^*C_{in}/^*C_{out})\}$ as a function of perfusion duration C_{in} and C_{out} represent nonradioactive Cl concentrations in the endolymph and perilymph respectively They are assumed to be 130 mM $^*C_{in}$ and $^*C_{out}$ represent radioactive ^{36}Cl concentration in the endolymph and perilymph respectively The regression line obtained by least square best fit has a slope of 0 01 an intercept of 0 0026 and correlation coefficient is 0 94 Numeral 2 indicates two overlapping points

...ssed by the following equation which been described in detail in the preceding (Konishi et al , 1978)

$$\frac{C_{in}}{C_{out}}\left\{1-\exp\left[\frac{-J}{V_{in}C_{in}}\ t\right]\right\}$$

$$\ln\left(\frac{C_{in}}{C_{out}}-\frac{^*C_{in}}{^*C_{out}}\right)=\ln\left(\frac{C_{in}}{C_{out}}\right)-\frac{J}{V_{in}C_{in}}\ t \quad (1)$$

=concentration of ^{36}Cl in endolymph
=concentration of ^{36}Cl in perilymph
=concentration of $^{-}$Cl in endolymph
=concentration of $^{-}$Cl in perilymph
= Cl flux (mM/min) across the endolymph–perilymph barrier
=volume of endolymph
=duration of perfusion

...re 4 shows the relationship between ln $_{in}/C_{out})-(^*C_{in}/^*C_{out})\}$ and duration of perfusion which is expressed by eq (1) C_{in} is y similar to C_{out} and both C_{in} and C_{out} remained unchanged regardless of the duration ...sion $^*C_{out}$ is assumed to be constant because of perfusion of perilymph By utilizing the least square best fit, the regression line can be expressed by

$$\ln\{(C_{in}/C_{out})-(^*C_{in}/^*C_{out})\}=0\ 0026-0\ 01t \quad (2)$$

The data were tested for linearity by using an analysis of variance technique (Dixon & Massey, 1957) and found not to differ significantly ($P=0\ 05$) from the above line The transport rate constant was 0 01 min^{-1} and the half time of exchange 69 3 min The line shown in Fig 3 was obtained from eq (2)

2 *Effect of anoxia*

Intravenous injection of a lethal dose of pentobarbital sodium resulted in a rapid decline of the cochlear potentials including EP EP changed its polarity and the mean value was −31 9 mV 20 min after perfusion was started

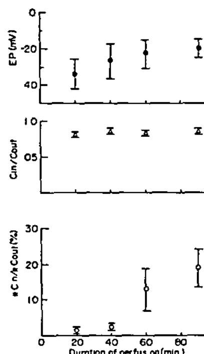


Fig 5 EP (uppermost panel), C_{in}/C_{out} (middle panel) and $*C_{in}/*C_{out}$ (bottom panel) as function of perfusion time in anoxic condition C and $*C$, total ⁻Cl concentration and ³⁶Cl concentration respectively, subscript *in* and *out*, endolymph and perilymph respectively Both scala vestibuli and tympani were perfused after animals re ived a lethal dose of pentobarbital sodium intra sly Three animals were used Vertical range bars standard deviations

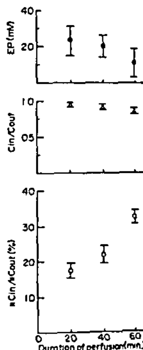


Fig 6 Effect of 10 ⁴M ouabain on EP (uppermost), C_{in}/C_{out} (middle panel) and $*C_{in}/*C_{out}$ (bottom Both scala vestibuli and tympani were perfused ³⁶Cl solution containing 10 ⁴M ouabain For were used for each perfusion time See caption

The EP gradually declined in magnitude as the perfusion proceeded, as shown in the uppermost panel of Fig 5

The concentration of ⁻Cl in the endolymph decreased in the anoxic condition The mean ⁻Cl concentration ratio of the endolymph to perilymph was 0 84 ($n=3$) with 20 min perfusion and its ratio remained little changed when the duration of perfusion was prolonged to 90 min (middle panel of Fig 5) The reduction of ⁻Cl concentration in endolymph during anoxia was also observed in the non perfused cochlea and the mean ratio of ⁻Cl concentration (endolymph to perilymph) was 0 85 ($n=5$) at 40 min anoxic period

The ³⁶Cl uptake in the endolymph was substantially decreased during the anoxic period As shown in the bottom panel of Fig 5, with 20 and 40 min perfusion ³⁶Cl uptake in the endolymph was only 1 5 and 2 5% of the concentration in the perilymph It increased to 19 2% when the duration of perfusion min

3 *Effect of ouabain*

The perfusion of the scala vestibuli and tympani with artificial perilymph containing M ouabain caused an irreversible suppression of the cochlear responses which was to that reported by Konishi & Mendelsohn (1970) In no case was a negative EP with 10⁻⁴ M ouabain and the rate of sion of the EP was generally slower than observed in anoxia (uppermost panel 6) The mean ⁻Cl concentration ratio lymph to perilymph) did not show a duction and was about 0 95 with 20 min sion ($n=4$) A slight decline of the concentration ratio was noted as the was prolonged The ³⁶Cl concentration endolymph with 20 min perfusion was

• II *Distribution of ^{36}Cl in perilymph and endolymph and EP under different conditions of* *sion in normal guinea pigs*[a]

perfusion		Perilymph (%)		Endolymph (%)	EP (mV)	n
ol	Cold sol	Scala vestibuli	Scala tympani			
T		100 8±7 8	100	28 6+1 2	82 4±2 3	5
		100	2 0±0 2	28 3+0 6	79 6±1 5	3
		90 7+14 5	100	15 7±0 6	79 3±1 6	3
	ST	100	0 5±0 3	33 0±1 8	81 0+1 7	3
	SV	3 7±2 7	100	4 2±1 0	82 3±2 2	4

ues are means±standard deviations n=number of guinea pigs Hot solution and cold solution artificial perilymph nd without ^{36}Cl respectively Duration of perfusion 40 minutes ^{36}Cl concentrations are normalized to perilymph scala to which hot solution was introduced

to that in the normal condition but with usion longer than 20 min suppression of uptake in the endolymph was noticeable 1 60 minutes perfusion the mean ^{36}Cl con-ration in the endolymph was 35 0±3 1% iat in the perilymph which was about 20% than the mean value obtained in normal nals

Perfusion of Scala Tympani vs Scala Vestibuli

temporal changes in the cochlear poten observed during perfusion of the scala pani alone were similar to those observed ing perfusion of both scala vestibuli and pani The changes in cochlear potentials ociated with perfusion of the scala vestibuli e in most cases less than those observed ing perfusion of the scala tympani

s described in the previous section the n ^{36}Cl concentration in the perilymph of scala vestibuli was slightly higher than that the perilymph of the scala tympani when h the scala vestibuli and tympani were per-ed for 40 min by introducing the perfusate) the scala tympani of the basal turn The an ^{36}Cl concentration in the endolymph was 6±1 2% (n=5) of that in the perilymph of scala tympani (Table II) The perfusion of scala vestibuli alone for a period of 40 min ulted in a mean ^{36}Cl concentration in the lolymph of 28 3±0 6% (n=3) which was nparable to that found in perfusion of both scala vestibuli and tympani However the ^{36}Cl concentration in the perilymph of the scala tympani was significantly lower (2 0±0 2%) The low activities of ^{36}Cl in the perilymph of the scala tympani are probably due to the rapid clearance of ^{36}Cl in the scala tympani as shown in Fig 2 The perfusate introduced into the scala vestibuli reaches the scala tympani through the spiral ligament but the clearance of ^{36}Cl is so fast in the scala tympani that no in crease in its concentration could be observed When the site of the perfusion was switched from the scala vestibuli to the scala tympani, the mean ^{36}Cl concentration in the endolymph was 15 7±0 6% (n=3) but the mean concen-tration in the perilymph of the scala vestibuli was 90 7±14 5% relative to the perilymph in the scala tympani

The results obtained in the separate perfu sion of the scala vestibuli or scala tympani were substantiated by a dual perfusion, simul taneous perfusion of the scala vestibuli with ^{36}Cl-Ringer's and perfusion of the scala tym-pani with nonradioactive Ringer's or vice versa When the scala vestibuli was perfused with ^{36}Cl-solution and simultaneously the scala tympani was perfused with Ringer's for 40 min, the mean EP at the time of endolymph collection was 81 0±1 7 mV (n=3) The mean ^{36}Cl concentration in the endolymph was 33 0±1 8% of that in the perilymph of the scala vestibuli The ^{36}Cl concentration was, of course, very low in the samples taken from

the scala tympani When the dual perfusion was carried out in the reverse manner, namely perfusion of the scala tympani with ^{36}Cl solution and the scala vestibuli with non radioactive solution for 40 min, the mean EP was *82 3±2 2 mV (n=4) and the* ^{36}*Cl concentration in* the endolymph was substantially lower with the mean value of 4 2±1 0% than that in the perilymph of the scala tympani

DISCUSSION

Our results demonstrate that the mean concentration of $^{-}$Cl in the endolymph and perilymph are 131 and 128 mM respectively and that there is no marked concentration gradient across the endolymph–perilymph barrier in the non perfused cochlea Our findings are in line with the previous reports (Smith, 1954, Rauch, *1964* Citron et al , 1956, Ledoux, 1950 and Bosher & Warren, 1971) The mean concentration of $^{-}$Cl in the endolymph and perilymph as increased to 139 0 and 138 9 mM respecvely by perfusing the perilymphatic space ith the artificial perilymph containing $^{-}$Cl concentration ranging from 140 to 160 mM However, it was noted that the perfusion of the perilymphatic space did not create a $^{-}$Cl concentration gradient across the endolymph–perilymph barrier In addition, perfusion did not result in marked suppression of the cochlear potentials It is safe to assume that the introduction of perfusate with $^{-}$Cl concentration ranging from 140 to 160 mM into the perilymphatic space does not greatly alter the physicochemical condition of the cochlea and does not modify the $^{-}$Cl transport process

*Sellick & Johnstone (1975) cited the un*published data of Robinson & Sellick, in which the scala media was perfused with low Cl sulfate Ringer's, producing an increase in EP of about 10 mV However the equilibrium potential for $^{-}$Cl changed from 0 to −92 mV (with 3 mM $^{-}$Cl concentration in the endolymph) The small effect on EP led them to assume that Reissner's membrane and other [illegible] cochlear partition are relatively [illegible] to $^{-}$Cl Kuijpers (1969) concluded [illegible] nificant contribution of $^{-}$Cl to EP [illegible] cluded on the basis of his experim[illegible] *which showed that a deficiency of* [illegible] perilymph affected EP to only a sl[illegible] The rate constant for $^{-}$Cl calculated [illegible] results was found to be 0 01 min^{-1} [illegible] ly smaller than the rate constant for [illegible] shi et al , 1978) Since the flux (J) is [illegible] uct of volume, concentration and ra[illegible] (see eq 1), the ratio of flux of Cl to [illegible] is found to be 0 67, when $^{+}$K and Cl [illegible] tration in the endolymph is assumed [illegible] and 130 mM respectively Similar [illegible] tions show that the flux of $^{-}$Cl across [illegible] lymph–perilymph barrier is much grea[illegible] flux of $^{+}$Na the flux ratio of Cl to $^{+}$Na [illegible] 60 to 90 ($^{+}$Na concentration in end[illegible] assumed to be 1 to 2 mM)

The results reported here show that [illegible] of ^{36}Cl in the endolymph is greater [illegible] sion of the scala vestibuli than with pe[illegible] of the scala tympani Therefore it is [illegible] Reissner's membrane is more perme[illegible] $^{-}$Cl than the rest of the cochlear parti[illegible] may argue the possibility of rupture [illegible] ner's membrane during the course of p[illegible] or collection of endolymph or perilymph [illegible] scala vestibuli If this is the case it [illegible] that the observed uptake of ^{36}Cl in the [illegible] lymph will be higher, because the end[illegible] would be contaminated by perfusa[illegible] possibility can be ruled out by the fact [illegible] cochlear responses to acoustic stim[illegible] decrease progressively during the pe[illegible] perfusion and the EP recorded at the [illegible] endolymph collection showed a sta[illegible] *ranging between 80 and 90 mV* Ther[illegible] results imply that Reissner's mem[illegible] more permeable to $^{-}$Cl than the rest of [illegible] dolymph–perilymph barrier Resp[illegible] vestigation carried out by Chou (19[illegible] a remarkable consumption of oxyg[illegible] ner's membrane Autoradiograp[illegible] tion of Reissner's membrane showed [illegible]

poration of leucine (Plester, 1960) Ilberg) injected a collodial suspension of thodioxide into the perilymphatic or endolear space and found that thorium dioxide sed Reissner s membrane in both direc-, by a combination of diffusion and an e transport mechanism Hinojosa (1971) rted that transport of ferritin takes place ss Reissner's membrane from the peri h toward the endolymph but not in the osite direction The mechanism underlying transport across Reissner's membrane is a matter of speculation in the present of our knowledge Further studies are ded to clarify the Cl transport mechanism eissner's membrane

ince no marked $^-$Cl concentration differ- was found between the endolymph and lymph in both non perfused and perfused hlea, the equilibrium potential due to $^-$Cl ived from the Nernst equation is roughly al to zero From the fact that the resting ue of EP ranges from 80 to 90 mV, it is ious that Cl is not passively distributed in cochlear fluids It is conceivable from our lts that the EP is a major driving force for ard movement of $^-$Cl from the perilymph ndolymph In anoxic conditions the magni of the EP decreases rapidly and usually polarity of EP changes to negative within a minutes after anoxia is induced The nega EP repels the $^-$Cl ions in the endolymph the perilymph, reducing the inward flux of l from the perilymph to endolymph This ounts for a decrease in $^-$Cl concentration in anoxic endolymph Instillation of 10^{-4} M bain into the perilymph resulted in supssion of CM and AP, and it reduced the nitude of EP At the 10^{-4} M ouabain con tration a negative EP was not observed in experiments The flux of ^{36}Cl from the rilymph to endolymph may be affected by slowly declining EP produced by 10^{-4} M bain to a lesser extent than by the rapidly ng EP produced by anoxia

order to maintain the balance of $^-$Cl con between the endolymph and peri lymph, it is apparent that $^-$Cl in the endolymph must be extruded from the endolymph Johnstone (1971) postulated several types of $^-$Cl transport mechanism and presented assumptions that $^+$K may be pumped in by an electrogenic pump and $^+$Na and $^-$Cl may be pumped by an electrically neutral pump The data presented here do not provide any conclusive evidence concerning the mode or site of the outward directed $^-$Cl pump The rate of ion transport is generally determined by the three factors, active transport, passive transport and solvent drag (Ullrich, 1974) In the steady state the net flux is zero and if the inward $^-$Cl flux from the perilymph to endolymph is assumed to be passive, then the outward flux is the sum of active transport and solvent drag It has been noted that solvent drag across epithelial tissues has been demonstrated convincingly only for relatively small, uncharged solutes (Leaf & Hays, 1962) At present it is difficult to evaluate precisely the physiological importance of solvent drag as a transport mechanism in the cochlea The solvent drag may represent a potentially important transport process that is as yet unexplored in the cochlea Further studies along this line are needed before the outward flux of Cl can be precisely evaluated

The dynamic equilibrium of electrolytes in the cochlear fluids is indispensable for maintaining the high sensitivity of the hair cells of the organ of Corti The understanding of the mechanism of homeostasis in the cochlear fluids is essential in studying the pathogenesis of various hearing impairments that may occur as a result of disturbance of the dynamic equilibrium of electrolytes in the cochlear fluids The results described here demonstrate our first step to elucidate the mechanism of electrolyte exchange between the perilymph and endolymph Our results indicate that the endolymph–perilymph barrier—especially Reissner s membrane—is permeable to $^-$Cl and the inward flux of Cl across the endolymph–perilymph barrier is passive, mainly driven by EP The $^-$Cl in the endolymph may be extruded

from the endolymph by an active transport mechanism or other process such as solvent drag

ZUSAMMENFASSUNG

Die Cl Überführung durch die Schranke der Endolymphe Perilymphe wurde bei Meerschweinchen durch Perfundieren des perilymphatischen Raumes mit kunstlicher Perilymphe die ^{36}Cl enthielt und Messung ^{36}Cl Aufnahme in die Endolymphe untersucht Bei normalen Tieren wurde kein bemerkenswerter Unterschied in Cl Konzentration zwischen Endolymphe und Perilymphe festgestellt Die Befunde zeigten daß die ^{36}Cl Aufnahme in die Endolymphe als einfache exponentielle Funktion der Perfundierungszeit darstellbar ist mit einer Geschwindigkeitskonstanten von 0 01 min $^{-1}$ Die Konzentration von ^{36}Cl in der Endolymphe war höher wenn die Scala vestibuli perfundiert wurde als wenn die Scala tympani perfundiert wurde was andeutet daß die Membrana vestibularis für Cl mehr durchlassig ist als die ubrige Schranke der Endolymphe Perilymphe Anoxie und ortliche Anwendung von Ouabain reduzierten die Konzentration von Cl und die Aufnahme von ^{36}Cl in die Endolymphe Unsere Ergebnisse lassen darauf schließen daß das endocochleare Potential die hauptsachliche Antriebskraft für den einseitig gerichteten $^{-}$Cl Fluß von der Perilymphe zur Endolymphe liefert

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OBSERVATIONS OF OTOCONIAL MEMBRANES FROM HUMAN INFANTS

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-ct The microdissection technique was used to otoconial membranes from 30 human infants ranging newborn to 2 years of age Both saccular and utric membranes were quite variable in overall shape the neonatal period the gelatinous layer of the membrane appears to thicken and become less to the macular surface than in the fetal period any infants older than 6 weeks otoconial membranes ound at autopsy to be completely dislodged from maculae with abnormally large saccular otoconia in four specimens Fourteen of the infants studied of sudden infant death syndrome and 16 died of r causes The incidence of detached otoconial mem was approximately the same in both groups Al the majority of these membranes were apparently odged post mortem the present findings suggest that onial membranes are susceptible to pathological al tion due to disease or head trauma

recent studies on inner ear pathology man have dealt with abnormalities of the conial membranes which cover the saclar and utricular maculae Johnsson & Haw s (1971, 1972) have, for example, dem a loss of both saccular and utricular conia in patients over 60 years of age Ross al (1976) utilized the scanning electron to study degenerative changes in conia from infants as well as from older uals In addition, Schuknecht (1974) provided evidence that dislodged otoconia y enter the posterior ampulla and provoke order termed 'cupulolithiasis' which is racterized by positional vertigo of the be paroxysmal type

he study of human otoconia has been y facilitated by the microdissection tech (Hawkins & Johnsson 1975) which al s otoconial membranes to be examined in tact and avoids decalcification procedures which radically alter the otoconial crystals

Accurate assessment of pathological alterations in human otoconial membranes depends upon recognition of artifactual changes which may occur during the postmortem period or during histological processing Knowledge of the normal range of morphological variation in these structures is also of importance in this regard Such data are, at present, quite limited

Our study is part of a continuing investigation of macular suprastructures in man dealing with the development of otoconial membranes, as well as their possible involvement in inner ear pathology This report focuses upon observations in human infants up to 2 years of age

METHODS

Inner ears from a total of 60 individuals were studied during the course of this investigation Thirty of these cases were fetuses obtained from premature deliveries which occurred during the third trimester of pregnancy or from prostaglandin abortions induced in the second trimester The other 30 cases were infants ranging from newborn to 2 years of age

Temporal bone specimens from the infants were removed at autopsy using a bone plug saw one inch in diameter Fetal specimens were cut from the skull floor with heavy surgical scissors The inner ears were fixed by perilymphatic perfusion utilizing either 1%

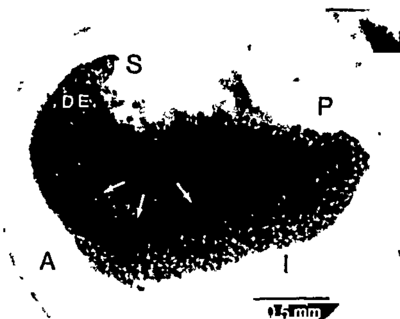


Fig 1 Surface view of s
otoconial membrane f
month-old infant Arrow
"snowdrift" line *DE* d
sion Anatomical orie
the skull indicated by
(anterior) *P* (posterior) *S*
and *I* (inferior)

osmium tetroxide or 2% glutaraldehyde Both fixatives were veronal-buffered at pH 7 4 When glutaraldehyde was used as the primary fixative the inner ear structures were stained vith 1% osmium tetroxide following overnight ıtaraldehyde fixation at refrigerator temrature After staining, specimens were partially dehydrated in ethanol and studied promptly to avoid possible artifacts due to prolonged storage

With minor modifications, all material was prepared for study by the microdissection technique as described by Hawkins & Johnsson (1975) The vestibule was opened by enlargement of the oval window and the saccular otoconial membrane photographed *in situ* with a Wild-Heerbrugg Model 7S stereomicroscope The anterior portion of the utricle containing the macula utriculi was then carefully dissected out and trimmed with iridectomy scissors to allow examination and photography of the utricular otoconial membrane

For scanning electron microscopy, otoconial membranes were removed from the maculae and placed on aluminum specimen holders They were then air-dried over silica gel, sputter-coated with gold and studied with a JEOL model 35 scanning electro scope operated at 15 kV

RESULTS

The surface morphology of the sacc conial membrane is illustrated in Fig specimen from a 9 month old infant formity with the shape of the saccul the otoconial mass is roughly oval with an extension at its anterior end jects dorsally A curved ridge know "snowdrift" line (Ades & Engstr Engstrom et al , 1966) is a promine feature of the crystal layer This band in the otoconial membrane lies the striola of the sensory epith deman, 1969)

A distinct "snowdrift" line is also on the surface of the utricular otoc brane as can be seen in Fig 2 specimen from a 16-month-old infant

Both the saccular and utricular of infants included in this study siderably in overall shape betwe individuals As illustrated in Fig



Fig 2 Utricular otoconial membrane from a 16-month-old female Snowdrift line is indicated by arrows *A* anterior *P* posterior, *L* lateral *M* medial

ions were particularly striking in saccular membranes, due largely to differces in configuration of the dorsal extension Otoconial membranes from fetuses and ne- were found to be tightly adherent to macular surface and therefore were very lt to dissect away from the sensory epielium intact In these specimens, the gela-us layer underlying the otoconia appeared be thinner and less cohesive than in older dividuals Otoconial membranes from in nts older than about 6 weeks could be dis off the maculae more easily and they perceptibly firmer in texture, even ugh they had been processed in the same ay as those from fetuses and younger infants

Altogether, 59 temporal bones from 30 innts were examined in the present study In of these the otoconial membranes were to be displaced from their normal posi in the saccule and/or utricle In each of ese cases it appeared that the membrane slipped from the macular area and crumpled into an irregularly shaped mass which was found lying unattached inside the saccule or utricle Typical examples of such detached otoconial membranes are shown in Fig 4

This phenomenon is definitely not due to fixation or storage artifact since it is possible to see the saccular macula clearly through the oval window and evaluate the condition of the otoconial membrane before fixative solution is introduced into the labyrinth It is usually also possible in unstained specimens to determine whether the utricular membrane is in place over the sensory epithelium

In four of the inner ears in which displaced membranes were present, the saccular otoconial mass contained very large crystals such as those illustrated in the scanning electron micrographs of Fig 5 Normal saccular otoconia from the infants included in this investigation were approx 4–15 μm in length, those from the membranes containing large crystals were up to 65 μm long and sometimes had abnormal shapes No abnormalities were found

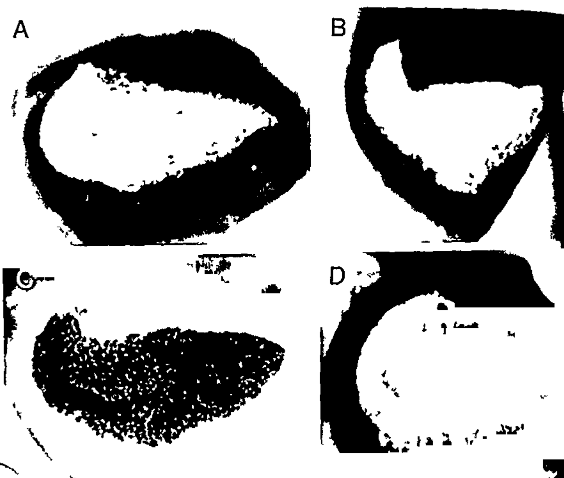


Fig 3 Variations in overall shape of saccular otoconial membranes from infants 3 months (A) 5 months (B) 11 months (C) and 24 months (D) of age

in the utricular otoconia from these specimens

Of the 30 infants whose inner ears we studied 14 were victims of sudden infant death syndrome (SIDS) and 16 died of readily identifiable causes In both these groups middle ear effusions were frequently found at autopsy Specifically 14 (52%) of the 27 SIDS specimens examined had effusions and 12 (38%) of the 32 temporal bones from infants who died of known causes were found to have middle ear effusions The middle ear mucosa was markedly hyperemic and edematous in all these cases regardless of the type of effusion present [clear fluid (serous) viscous yellowish mucous (mucoid) or pus (purulent)]

Specific data regarding the condition of otoconial membranes presence of middle ear effusions age and approximate post mortem time before fixation are given in Tables II and III

Since our observations on neonatal otoconial membranes suggest that they have physical characteristics much like those of fetuses the data on infants up to 6 weeks of age are presented separately in Table I Detached membranes were present in 2 (1[illegible]) of the 16 specimens in this group The incidence is similar to that found in second and third trimester fetuses dislodged otoconial membranes were present in 6 of the 6[illegible] ears in our collection

As Tables II and III show displaced membranes were found with much greater

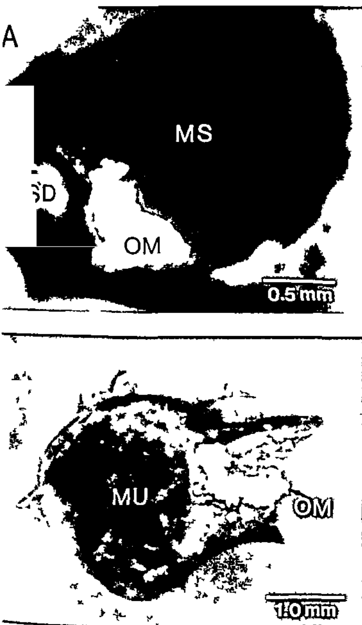


Fig 4 (A) Displaced crumpled saccular otoconial membrane (OM) from a ? month-old infant The letters MS are placed in the central port on of the darkly sta ned sac cular macula *SD* saccular duct (B) Utricular macula (*MU*) from the same infant show ng detached otoconial membrane (*OM*) lying near med al aspect of macula Note d fference in scale relat ve to (A)

quency in older infants Also the incidence was approximately the same in SIDS victims as in children who died of other causes 70% n the SIDS group and 75% in the other cases We believe this finding is related to a develop al change occurring during the first sev weeks of life which renders the otoconial membrane more susceptible to displacement he actual detachment of membranes from the macular areas may be caused by a variety of factors some of which will be considered in the following discussion

DISCUSSION

The present investigation of inner ears from human infants revealed marked variations in the shape of otoconial membranes between



Fig 5 (A) Normal saccular otoconia from a 2 year-old female infant (Case 44 right) (B) Giant saccular otoconia from a 6-month-old infant (Case 37) The largest crystals seen in this view are approximately 40 µm long otoconia up to 65 µm in length were present in other areas of the specimen (C) Very large saccular oto[illegible] month-old infant (Case 24) Note dense [illegible] like packing and peculiar shape of some crystals [illegible] nification (indicated by bar at lower right in [illegible] each micrograph

ıble I *Data on infants up to 6 weeks of age*

st column identifies major pathology "+" indicates intact otoconial membrane, "0" detached membrane Ab-viations PMT, post mortem time, S saccule, U, utricle, R, right, L, left, Bilat, bilateral

se	Age	PMT (hours)	Right ear S	Right ear U	Left ear S	Left ear U	Middle ear effusion
SIDS	6 weeks	6	+	+	+	+	R, none, L, serous
SIDS	6 weeks	21	0	0	+	+	Bilat serous
Sepsis	1 week	15	+	+	+	+	None
Coarctation of aorta	1 day	17	+	+	+	0	None
Hydrocephalus	newborn	14	+	+	+	+	None
Omphalocele	6 weeks	8	+	+	+	+	None
Aortic atresia	4 days	24	+	+	+	+	None
Coarctation of aorta	6 weeks	20	+	+	+	+	None

able II *Data on infants who died of causes other than SIDS*

ajor pathology identified in first column Symbols and abbreviations as in Table I

ase	Age (months)	PMT (hours)	Right ear S	Right ear U	Left ear S	Left ear U	Middle ear effusion
Sepsis	16	8	+	+	+	0	None
Encephalitis	4	11	0	0	0	0	R, mucous, L, purulent
Cerebral hemorrhage	11	8	+	0	+	0	Bilateral purulent
Asphyxiation	2	4	0	0	+	+	Bilateral serous
Sepsis	18	22	0	0	0	0	None
Burn victim	24	8	+	+	0	0	None
Asphyxiation	5	14	0	0	0	0	None
Asphyxiation	4	13	+	+	+	+	R, mucous L, purulent
Asphyxiation	7	12	0	0	0	0	Bilateral purulent
Fatty liver	3 5	4	0	+	0	+	R, mucous, L, purulent

able III *Data on SIDS victims*

ymbols and abbreviations as in Table I Only the left ear from Case 2 was available for study

ase	Age (months)	PMT (hours)	Right ear S	Right ear U	Left ear S	Left ear U	Middle ear effusion
2	3	10			0	+	None
1	2	5	0	0	0	0	None
4	6	12	0	0	0	0	Bilat purulent
5	2	6	0	0	+	0	None
7	4	6	+	+	+	0	None
0	3	6	+	+	+	+	R purulent, L, none
2	9	19	0	0	+	0	Bilat purulent
3	3	12	+	+	0	0	None
5	2	10	0	0	0	0	Bilat serous
6	3	6	+	+	+	+	None
7	6	21	0	0	0	0	Bilat purulent
8	5	10	0	0	+	+	Bilat purulent

different individuals This variability is particularly striking in saccular membranes where obvious differences in the size and shape of the dorsal extension are readily observable

In common with fetal otoconial membranes, those from infants up to about 6 weeks of age were firmly adherent to the macular surface Otoconial membranes from older infants appeared to have a thicker, more cohesive gelatinous layer They were much easier to dissect away from the maculae intact

This maturational change in the physical character of the otoconial mass is apparently of significance in accounting for our finding that many otoconial membranes in older infants were completely detached from the vestibular maculae at the time they were first examined, before the inner ears were perfused with fixative

Fourteen of the 30 infants whose temporal bones were included in this study were victims of sudden infant death syndrome, in the remaining cases death was attributable to accidental causes or to specific pathological lesions Dislodged otoconial membranes were found with about equal frequency in both these groups of infants

In accordance with previous studies of neonates and infants (McLellan et al, 1962, Paradise et al, 1976), we noted a rather high incidence of middle ear effusions in the present material Effusions were found about 14% more frequently in the SIDS group This is not particularly surprising in view of the fact that mild infections, especially of the upper respiratory tract, have been reported in a high percentage of SIDS victims (Naeye, 1973, Naeye et al, 1976)

Arnold et al (1977) have provided evidence that chronic mucous effusions in children can produce a sensorineural hearing loss which they believe is due to a reduction in oxygen supply to the inner ear and to the influence of histamine released by the metaplastic epithelium of the middle ear An increased incidence of sensorineural hearing loss in patients with chronic purulent otitis media is also well documented (Paparella et al 1970) regard to the vestibular end organs knecht (1974) has noted a positive corre between the presence of otitis media a disorder known as cupulolithiasis which lieved to involve dislodged otoconia T seems possible that biochemical alterat the inner ear fluids (associated with mid effusions) might produce pathological c in the otoconial mass in infants

It is interesting to note at this point each of the three cases in our series giant saccular otoconia were found b otitis media was also present The larg tals were definitely not artifacts due to f or storage and they seem unlikely to result of post mortem autolysis sin utricular otoconia were normal in thes imens In relation to the specific eff autolysis on the otolith organs the work of Ross et al (1976) is also per Using Rhesus monkeys as experimenta els, they found that the otoconial mem remained intact and contained normal c after post mortem times as long as 72 h

On the basis of data collected thus fa ever, there appears to be little eviden middle ear effusions contribute signif to the actual displacement of otoconial branes Among the cases listed in Table incidence of detached membranes is th in those with middle ear effusions as i free of effusions In the SIDS group III), 9 of the 11 temporal bones with eff also had dislodged membranes while in 12 ears without effusions displaced oto membranes were found

Certainly, one of the greatest difficul the study of anatomical materials obta autopsy is the evaluation of effects pr by post mortem autolysis In the present this is especially true of specimens fr SIDS cases since only a rough approxi of the time delay between death and fi of the temporal bones can be made

We can, however, divide these case two subgroups on the basis of relative

ɔt mortem time If this is done, it is found it displaced otoconial membranes were in 5 (50%) of the 10 ears with post-rtem times of less than 10 hours At long ɔt mortem times the incidence is consider-ly higher 11 (85%) of the 13 SIDS speci-ns with post mortem times of 10 hours or re had dislodged membranes The data m infants who died of other causes shows incidence of 70% at post mortem times be 10 hours and 80% at 10 hours or more

Thus in both groups of infants, detached conial membranes were found more fre-y in material in which the post mortem ay was long As Table I shows, the 2 unger infants with dislodged membranes o had post mortem times close to 20 hours should be noted, however, that in some in nces (e g case 21) all four membranes were tached even though the post mortem time s relatively short and in others (such as case) the otoconial membranes were intact in ite of long post mortem times

Finally, vibration of temporal bone speci by the oscillating saw used during ex-ction from the skull may dislodge the al mass in some cases Interestingly ough vibration from the saw was evidently t often severe enough to dislodge mem in infants less than 6 weeks old Also tion cannot be the sole cause for mem ne displacement since detached mem were occasionally found in fetal speci which were removed from the skull with ssors

n conclusion, our observations suggest a nuance of developmental change in hu n otoconial membranes after birth During neonatal period, the gelatinous layer of the conial mass appears to thicken and separ-from the macular surface to the extent that entire membrane may be dislodged rather sily from the macula Approximately three-arters of the 43 ears studied in infants older an 6 weeks contained saccular and/or utric ar otoconial membranes which were com letely detached from the maculae

Complete membrane detachment apparently occurs during the post mortem period in most cases This phenomenon is therefore not necessarily indicative of pathology Otoconial membranes may be dislodged as a conse-quence of mechanical vibration during autopsy procedures, or as the result of autolysis when the delay before fixation of specimens is long

On the other hand, the results of this study do suggest that otoconial membranes are sus-ceptible to pathological involvement in the presence of ear disease or head trauma This is particularly true for older infants in whom the membranes are more loosely attached to the sensory epithelia These findings therefore point toward a need for reassessment of the more traditional concept in which the macular suprastructures have been considered to be static and invariable after the time of birth

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ZUSAMMENFASSUNG

Mittels Mikrosezieren sind die Otolithenmembrane von 30 Kleinkindern im Alter von neugeboren bis zwei Jahre alt untersucht worden Sowohl die Sacculusmembran als auch die Utriculusmembrane waren sehr unterschied lich in ihrer Form Im Vergleich zur Fotusphase scheint es daß in der Neugeborenenphase die gelatineartige Schicht der Otolithenmembran sich verdickt und sich etwas von der Maculaflache lost Bei vielen Sauglingen die alter als sechs Wochen waren zeigte die Autopsie daß die Otolithenmembrane sich ganzlich von der Macula gelost hatten und in vier Fallen fand man anormal große Sacculusotolithenchrystalle Vierzehn der untersuchten Sauglinge starben an dem sudden infant death syn drome (plotzliches Sauglingssterbesyndrom) und sech zehn starben durch andere Ursachen Die Zahl der los gelosten Otolithenmembrane war für beide Gruppen fast gleich groß Obwohl die Mehrzahl dieser Membrane sich scheinbar erst postmortem gelost hatten lassen die bis jetzt vorliegenden Untersuchungsergebnisse vermuten daß bei Krankheiten oder Kopftrauma die Otolithen membran zu pathologischen Veranderungen neigt

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FURTHER STUDIES OF THE EFFECTS OF CONTINUOUS WHITE NOISE OF MODERATE INTENSITY (70–80 dB SPL) ON THE COCHLEA IN YOUNG GUINEA PIGS

Time Course and Distribution of Hair Cell Degeneration

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stract Guinea pigs aged one week were exposed to te noise at a maximum of 76 dB SPL for 7 days and re then killed 3 8 and 16 weeks later for histological amination of the cochlea by the surface preparation hod Appreciable increases in outer hair cell losses re observed in the apical turn 3/3½ chiefly in the outer o rows between the 3rd and 8th week but not be-een the 8th and 16th week No significant losses were en in control groups corresponding to the 3 and 8 week nods although in the control group for 16 weeks sur ival small deficits attributable to natural ageing were in the apical half turn 3½

he question of whether exposure to exces-ive hospital incubator sound is likely to cause _ losses in premature babies has been he subject of some discussion (e g Seleny & 'yn, 1969, Blennow et al , 1974, Falk et , 1974) It is well established that young ani-ls are, in general, more susceptible than old to high intensities, that is, about 120 dB et al , 1974, Coleman, 1976b), and re / we have shown that continuous white noise as low as 70–80 dB, an amplitude quite in hospital incubators, can cause le damage to the cochleas of very _ _a pigs (Douek et al , 1976) In our udy we assessed cochlear damage by the sur- preparation method (Engstrom et al ,) allowing 3 weeks to elapse between the d of acoustic stimulation and histological -mination to permit damaged hair cells to egenerate However, in a later electron croscope study of the process of degenera- (Dodson et al , 1977) we have found evidence that degeneration of hair cells continues well beyond 3 weeks after experimental stimulation, suggesting that the final level of damage might be considerably greater than that seen in our previous study Similar long term effects have also been detected electron microscopically by Spoendlin & Brun (1973), although little is known about the quantitative aspects of such changes or indeed about the time course of hair cell degeneration after any type of trauma

It was therefore decided to determine the final extent of hair cell loss in young guinea pigs, after a continuous, 1 week white noise exposure and to establish the characteristic pattern of deficits in the different regions of the cochlea In addition, since the number of lost hair cells was not great in our previous study, it could be objected that such deficits might be caused by uncontrolled noise in the animal house in which the guinea pigs were kept, or, in the long term experiments, they might be caused by natural ageing processes, accordingly, further controls have been carried out to evaluate the contribution of such factors to the observed damage

MATERIALS AND METHODS

Groups of guinea pigs (Dunkin Hartley strain) aged 1 week were placed with their mothers in an acoustically insulated box fitted with two

Table 1 *An analysis of outer hair cell losses in experimental and control groups in this investigation*

On the left the cochlear half turns of animals surviving for 3 8 and 16 weeks after treatment are compared, on the right a similar comparison of the control groups of corresponding ages is shown
Abbreviations N S not statistically significant OHC outer hair cell

Experimental groups				Control groups		
Cochlear region	Survival (weeks)	Mean and S D (% OHC loss)	*P*	Age (weeks)	Mean and S D (% OHC loss)	*P*
3½	3	10 4±6 0	<0 05>0 02	5	1 1±0 9	N S
	8	18 7± 7 5	N S	10	0 8±0 7	<0 01
	16	16 7±10 7		18	5 6±1 1	
3	3	3 6± 3 3	<0 02>0 01	5	0 8±0 7	N S
	8	12 0± 7 2	N S	10	1 3±0 8	<0 1>0 05
	16	15 4±11 2		18	3 4±1 6	
2½	3	2 3± 3 1	N S	5	0 4±0 4	N S
	8	3 0± 2 4	N S	10	1 2±0 6	N S
	16	10 1±11 4		18	2 3±1 3	
2	3	2 6± 2 6	N S	5	0 3±0 4	N S
	8	0 5± 0 4	<0 05>0 02	10	0 3±0 3	N S
	16	1 0± 0 4		18	0 4±0 3	
1½	3	1 4± 1 5	N S	5	0 6±0 7	N S
	8	0 2± 0 2	<0 01>0 001	10	0 6±0 7	N S
	16	1 5± 0 6		18	0 7±0 9	
1	3	1 9± 4 5	N S	5	0 5±0 8	N S
	8	0 1± 0 1	N S	10	0 1±0 1	N S
	16	0 2± 0 5		18	2 4±5 0	
½	3	0 1± 0 1	N S	5	0 1±0 1	N S
	8	0 1± 0 1	N S	10	0 0±0 0	N S
	16	0 1± 0 1		18	0 4±0 6	

loudspeakers connected to a white noise generator, and were subjected to continuous white noise for 7 days A frequency spectrograph of the acoustic stimulus, measured with a Bruel & Kjær sound level meter inside the box, is shown in Fig 1 It will be noted that there are two maxima, one at 500 Hz and the other at 4 000 Hz, both of 76 dB

After acoustic stimulation, animals were kept for 3, 8 or 16 weeks and were then killed with an overdose of barbiturate decapitated and their cochleas fixed in 3% phosphate buffered glutaraldehyde (pH 7 4 0 1 M) for 4 hours, small holes being made in the cochlear bone to admit the fixative The cochleas were then washed in buffer, postfixed in buffered 1% osmium tetroxide and partially dehydrated in 70% ethanol Pieces of spiral organ were dissected out, then mounted in glycerol Hair cell losses, observed by phase contrast microscopy, were counted and recorded in the form of cochleograms following the method of ... et al (1974) and Coleman (1975) in each half turn, cell losses in 3 rows of 100 outer cells (i e 300 in total) were noted and expressed as a percentage of cells in that region

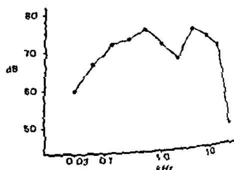
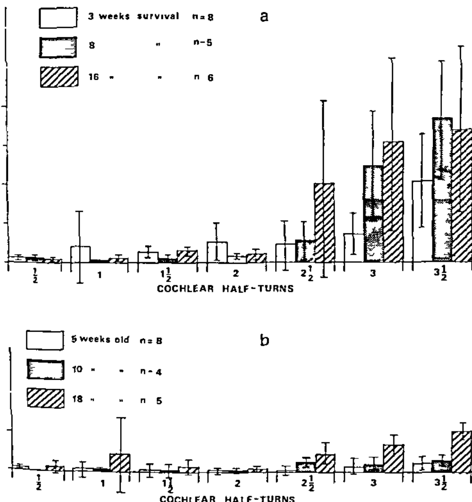


Fig 1 The frequency spectrogram of the white stimulus used in this study as measured inside the box



2 Histograms depicting the distribution of outer hair losses in experimental and control groups of guinea In (*a*) different regions of the cochlea are compared 8 and 16 weeks after acoustic exposure In (*b*) con- l groups of ages corresponding to those of the three experimental groups are shown The histogram blocks represent mean values and the error bars represent standard deviations *n* is the number of animals in each sample

no inner hair cells were found to be issing, these were not included

Control groups of similarly aged guinea pigs kept alongside the experimental groups the duration of the survival time to see if able hair cell losses resulted from ral ageing or uncontrolled environmental during this period These control ani- s were killed at the same time as their ex- ental counterparts The actual ages of all s were of course 2 weeks more than the ntal survival period, since they were already one week old at the beginning of the experimental treatment which itself lasted for 1 week

For statistical purposes each animal was taken as a single sample, the number of lost hair cells being the mean of those of the cor responding regions in the two cochleas (fol lowing Coleman, 1976*a*) Sample sizes are shown in Fig 2 As an examination of the hair cell losses in both the experimental and control animals showed that they followed an approximately normal frequency distribution,

Table II *An analysis of the ratio of outer hair cells lost in each of the three rows in turn] expressed as a percentage of the total outer hair cells lost in this region*

Outer hair cell row	Experimental survival time 3 weeks	8 weeks	16 weeks	Controls age 18 weeks
1	9 6±17 0	3 3± 3 0	8 8±12 2	10 7±11 8
2	29 3±38 1	35 2±25 5	35 9±25 2	23 2± 8 7
3	61 0±38 2	61 4±23 8	55 3±35 4	66 1±32 8

the Student's *t*-test was used to estimate the significance of hair cell deficits at different survival times and ages In a number of cases the results of this statistical analysis were compared with those of a non parametric method (the Wilcoxon's rank sum or Mann-Whitney U-test) but no significant differences between the results of the two methods could be demonstrated

RESULTS

The losses of outer hair cells in the spiral organs of 1-week old guinea pigs exposed to 7 days' continuous white noise and killed or 16 weeks later are shown in Table I and 2*a* For comparison, hair cell losses in control groups are shown in Table I and 2*b* Data for the 3 week experimental and trol group are taken from our previous s (Douek et al , 1976)

It is evident that the total numbe of cells lost *in the 8 weeks* survival gro significantly higher than in the 3 weeks g the further degeneration being partic noticeable in half turn 3 and to a lessere *in half turn 3½* However, between the 8 a weeks' survival groups no further signi

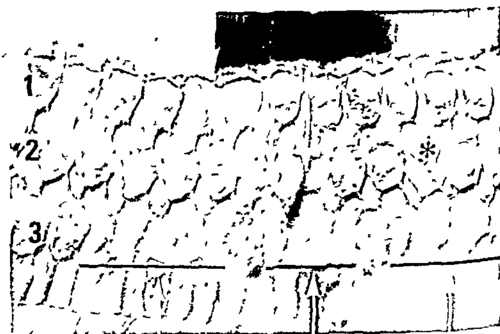


Fig 3 Micrograph of part of a surface preparation of half turn 3 eight weeks after exposure to 7 days continuous white noise showing areas of damage in the three rows of outer hair cells (the numbers designate the rows) Note the presence of severe damage in row 3 moderate damage includ ng the recent formation of a phalangeal scar 2 and a single phalangeal scar in row 1 M ss ng c ind cated by arrows and the recent scar by an a Interference-contrast microscopy

ase is detectable except marginally in half , 2 and 1½ where the numbers lost are in case negligible

the control groups, no significant hair cell s were observed in animals aged 10 weeks esponding to the experimental 8 weeks' ival group) in comparison with the 5-k-old animals, but in the 18 week old con group low but significant deficits had oc ed in turn 3/3½

he majority of hair cell losses in experi tal animals were in the outer two rows of outer hair cells following the pattern simi o that seen in natural ageing (Table II and 3)

DISCUSSION

se results confirm and extend our previous lusion that continuous white noise in the 30 dB range is injurious to the cochleas of ng guinea pigs (see Introduction) The nce of marked effects in the control group ws that the losses in the experimental ani s are due to exposure to this stimulus ler than to ageing or uncontrolled environ ntal noise, the notable increase in hair cell icits in the cochlear turn 3/3½ between the nd 8 weeks' survival groups, where finally sixth of the outer hair cells had de ierated shows that the effect is quite promi it even at such low sound intensities

he distribution of damage along the spiral an in the sound treated animals is similar that seen in young guinea pigs exposed to longed periods of white noise at much her intensities (about 120 dB) reported by lk et al (1974) and it is reasonable to sup e that the type of trauma is essentially simi

The distribution of losses between the ee rows of outer hair cells is also the same that seen in other types of acoustic damage Coleman, 1976) The threshold for oustic damage therefore appears to be con ierably lower in young animals than in ults in which 90 dB is usually taken as the lower limit (Spoendlin 1976), and this finding emphasises the risk of exposing new born in fants to constant noise even as low as 76 dB

These results also show that at least in young animals, 3 weeks is too short a period for the complete degeneration of affected cells and that 8 weeks' survival is a more satisfactory interval for this purpose There appears to be no virtue in extending the period beyond this time, since no appreciable increase was seen at 16 weeks and at this time natural ageing processes begin to contribute signifi cantly to hair cell losses, cell deficits seen in the 18 week old control animals are similar in size to those observed at about this age by Coleman (1976)

The reasons for the observed increase i hair cell deficits in the 3–8 week period are not clear from these experiments Continued losses observed in this interval could be a con sequence of variable rates of collapse and dis appearance of cells killed by the initial stimu lus, or perhaps of an increase in the suscep tibility of mildly damaged cells to environ mental noise A third possibility is that some other component of the cochlea e g the stria vascularis, was injured by excessive acoustic exposure, leading to a general disturbance of cochlear homeostasis during this time

The restriction of damage to the apex of the cochlea is also interesting since it might be ex pected from the bandwidth of the white noise and its frequency distribution that damage would also be found in more basal parts of the cochlea Coleman (1976) for example reported that acoustic over stimulation with a 4000 Hz tone at 119 dB caused maximum destruction in the region of the basal turn We have re cently observed by means of transmission and scanning electron microscopy that the cells of the apex in 1 week old guinea pigs show many signs of immaturity when compared with those of more basal regions (Dodson et al 1977) so that the distribution of damage may be related primarily to the developmental pattern of the cochlea rather than to its mechanical prin ciples

ACKNOWLEDGEMENTS

We wish to thank the Medical Research Council for supporting this study financially We are also grateful to Mr R Cunnell for constructing the sound box to Mr Keith Humphries for technical assistance, to Dr Rosemary Dalton for statistical advice, and to Dr Ade Pye for instruction in the surface preparation method

ZUSAMMENFASSUNG

Junge Meerschweinchen wurden mit white noise (70–80 dB SPL) 7 Tage lang beschallt Drei, 8 oder 16 Wochen nach der Beschallung wurden die Tiere getötet und die Cochlea wurde histologisch untersucht Die Verlustrate von Haarzellen in den apikalen 3/3½ Schneckenwindungen war deutlich erhoht nach 3 bis 8 Wochen Uberlebenszeit aber nicht nach 8 bis 16 Wochen Hauptsachlich die äußeren zwei Reihen waren betroffen Keine signifikanten Verluste wurden in den Kontrollgruppen (3–8 Wochen) beobachtet In der Kontrollgruppe (16 Wochen) wurden kleine Verluste in der apikalen (3½) Windung beobachtet die auf Alterung zuruckzuführen waren

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A PHYSIOLOGICAL AND MORPHOLOGICAL STUDY OF THE COCHLEA OF THE RAT FOLLOWING TREATMENT WITH ATOXYL AND NEOMYCIN

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ct By means of physiological and morphological iques the inner ear pathology following exposure to totoxic compounds atoxyl and neomycin was ana l in the rat Primarily a high tone deterioration oc d with a subsequent morphological degeneration pat among the hair cells in the basal part of the cochlea outer hair cells were more frequently affected than nner hair cells following the administration of both d and neomycin

the normal and the pathological function e labyrinth have been studied in a large ety of animal experiments concerning the ology and the morphology of the inner (Keidel & Neff, 1976, Smith & Vernon, The guinea pig has been the most wide-sed experimental animal though also the the chinchilla, the cat and others, and ntly the lizard have also been studied Sjoback & Wersall, 1973, Bagger Sjo 1977)

the evolution of inner ear changes fol ing both ototoxic stimuli and aging, the cell degeneration pattern has been rather In the cochlea the earliest and st changes occur among the hair cells of basal coils (McGee & Olszewski, 1962 onen 1965, Elliott & McGee, 1965 Lundquist & Wersall 1966 1967, Engstrom et al, 1966, Stebbins et al, 1969, 1973, Ylikoski, 1974) Thus Hawkins (1973) stated "that the hair cells of the cochlea react in a graded, sequential and stereotyped fashion to insults that exceed their tolerable limit, regardless of the immediate cause of injury"

The atoxyl induced sequence of hair cell damage appears to be in contrast to the general pattern and can be selectively limited to the apical part of the guinea pig cochlea (Anniko, 1976) The mechanism of this effect is, however, not known

Lesions restricted to the apical coils so far have been reported only following experimental obstruction at various levels of labyrinthine blood vessels (Kimura & Perlman 1958, Bernstein & Silverstein, 1966 Alford et al, 1965) and in cases of idiopathic labyrinthine hydrops (Kohut & Lindsay, 1972) However, the pathological changes in the inner ear of such lesions were not restricted to the hair cells only but involved also other structures of the membranous labyrinth

The aim of the present investigation was to study whether or not the atoxyl induced location of hair cell damage is specific to the guinea pig or if this pattern can be reproduced also in other species, e g the rat as used in

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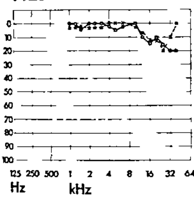
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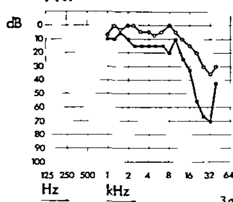
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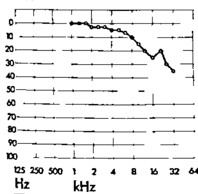
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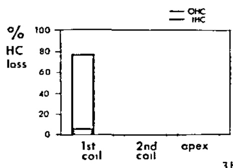
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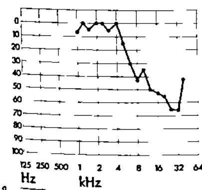
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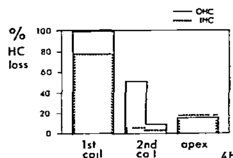












Figs 1 7 9 11–12 N_1 N_2 threshold shift curves The divergence measured in dB in the experimental animals of the threshold shift relative to the normal threshold was registered as hearing impairment (vertical axis) The investigated frequencies were marked on the horizontal axis A atoxyl N neomycin Fig 1 Animal no A 23 ×—× left ear O—O right ear

Fig 2 N_1 N_2 threshold shift curve Animal no A 7 O—O right ear

Fig 3 Animal no A 19 (*A*) N_1 N_2 threshold shift curve ■—■ left ear O—O right ear Differences occur in threshold shift between inner ears of the two sides (*B*) Histogram illustrating cochlear hair cell loss of the left ear The sensory cell damage was primarily confined to the outer hair cells of the basal half of the 1st coil

Fig 4 Animal no N 11 (*A*) N_1 N threshold shift curve ●—● right ear (*B*) Histogram illustrating the extent of hair cell damage of the right ear in various coils of the rat cochlea

Morphological preparation technique

Following decapitation of the animal the inner ear was explored via the bulla tympanica after removal of the auditory ossicles The stapes was extracted, the round window membrane removed and a hole was made at apex and to the lateral (horizontal) semicircular canal The labyrinth was locally perfused with a 2% osmic acid solution in Veronal acetate buffer (pH 7 2–7 4), a technique originally described by Wersall (1956) concerning the guinea-pig

The specimens remained in the fixative for 2 hours and were thereafter treated according to standard methods for biological electron microscopy (Hayat, 1970) and embedded in Epon (Luft, 1961) Embedded cochleae were divided along the mid modiolus, sectioned and mounted for mapping of the cochlear hair cells, according to the surface preparation technique for cytocochleograms (Ernstsson, 1972, Anniko, 1976) Representative areas from the surface preparations were investigated by light microscopy (staining toluidine blue) and electron microscopy (Anniko & Wersall, 1975)

RESULTS

Individual differences occurred among animals in susceptibility to atoxyl and neomycin with regard to N_1 N_2 threshold shift as well as to morphological change The animals were in good clinical condition at the time of sacrifice The animals treated with atoxyl showed a normal weight increase during the treatment period, while those treated with neomycin showed a less than normal weight gain during that period The animals were grouped with regard to the slope of the N_1, N_2 threshold shift curve in three subgroups independent of the drugs administered and the dose

Minimal threshold shift

This group comprises animals with a 10–30 dB threshold shift over one or two octaves in the frequency range studied (1–40 kHz) The animals receiving 50 mg/kg of atoxyl all belonged to this group (10 rats) together with one rat treated with 75 mg/kg of atoxyl (total d 350–650 mg/kg vis à vis 375 mg/kg) threshold shift occurred without exception the octave bands 8–32 kHz (Fig 1) and similar in both ears

The difference in threshold shift betw animals receiving the same total amour atoxyl was only minimal when investigate 45 or 62 days following the last administra

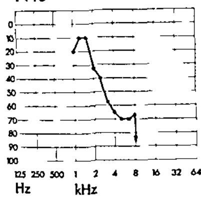
The morphological analysis of surface p arations revealed a uniform outer hair (OHC) damage pattern among the variou mals belonging to this group which sh only a minimal (1–4 8%) loss of OHC r evenly distributed in the basal coil Bot outer and the inner hair cells (IHC) of th cal region and the upper part of the 2n appeared intact In general the histol sections showed a normal structure The were intact in all but one of the invest cochleae (A 7)

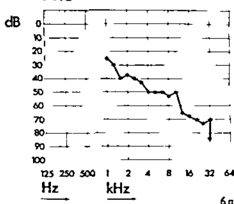
One animal (A 7), however, showed most 80% degeneration of OHC in the part of the 1st coil, though most of the were well preserved (Fig 2) In contras upper half of the basal coil showed a limited hair cell damage—less than a fe cent There was only a small threshol amounting to 35 dB at 31 5 kHz whi other frequencies were considerably le fected (10 kHz, 15 dB 12 kHz 20 dB 1 25 dB, 20 kHz 20 dB and 25 kHz 3 Two animals receiving 50 mg/kg of ato each occasion (totally 350–400 mg/kg h threshold shift and their cytocochleo showed loss of only single OHC in the coil in no part exceeding 1–3%

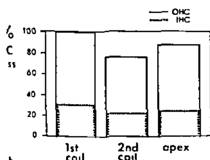
None of the neomycin treated anima longed to this group with minimal chan the hearing threshold

Sloping threshold shift

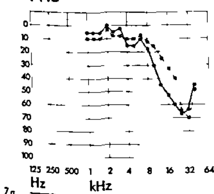
This classification was used when the t old shift was at least 30 dB in a freq range of 1 or 1½ octaves Both atoxyl an mycin treated animals revealed this t hearing impairment











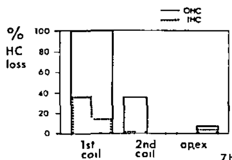


Fig. 5. N_1–N_2 threshold shift curve. Animal no. N 16, right ear. No electro-physiological response was found concerning frequencies above 8 kHz.

Fig. 6. Animal no. A 10. Right ear. (*A*) N_1–N_2 threshold shift curve ●—● right ear. (*B*) Histogram illustrating severe degeneration of outer hair cells at all levels of the cochlea while many inner hair cells still were identified in the surface preparations. (*C*) Light microscopy (LM). Degeneration of outer and inner hair cells. The structural organization with an inner and an outer tunnel is still preserved.

Fig. 7. Animal no. N 15. (*A*) N_1–N_2 threshold shift curve ●—● right ear, ■—■ left ear. (*B*) Histogram of the right ear illustrating severe degeneration of outer hair cells in the basal part of the cochlea. The inner hair cells appear considerably more preserved even in the basal coil. (*C*) Interference contrast light microscopy. Surface view from the basal part of the second coil showing damage to outer hair cells in all three rows (OHC I–III) while the inner hair cells (IHC) all are preserved at this distance.

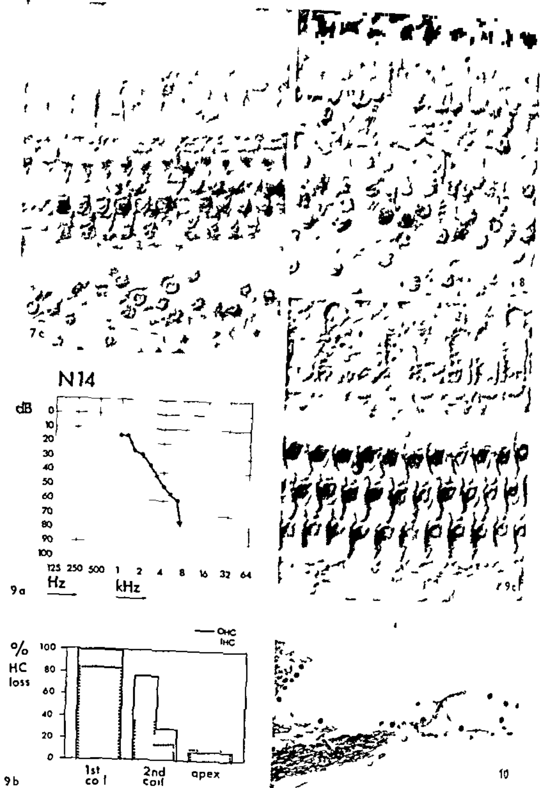
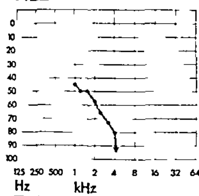
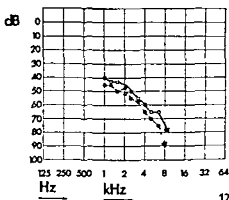


Fig 8 Interference contrast l ght m croscopy Animal no N11 Basal co l Total degeneration of outer and inner hair cells

Fig 9 An mal no N14 Right ear (*A*) N N_2 threshold sh ft curve ●—● right ear An impairment of hearing was found concerning the high frequencies No detectable hearing could be recorded in frequenc es above 6 3 kHz (*B*) H stogram illustrat ng severe damage to the hair cells of the organ of Corti in the basal and m ddle parts of cochlea (*C*) Interference contrast l ght m croscopy N mal outer (OHC I–III) and inner (*IHC*) hair cells in apical half of the second co l

Fig 10 LM Animal no N24 2nd coil All hair cells degenerated and the structural diffe ent at on of s port ng cells is lost





11 N_1 N_2 threshold shift curve Animal no A22 right ear Severe hearing loss
12 N_1 N *threshold shift curve Animal no N24* ●—● right ear □—□ left ear The degree of hearing impairment is roughly the same in the inner ear on both *sides*

This group consisted of totally 14 animals, alf of which belonged to the atoxyl—(75 mg/ g b w on each occasion except one rat— 10—which received 125 mg/kg/day three mes) vis a vis neomycin treated (100 mg/kg n each occasion) rats The atoxyl induced ld shift mainly involved frequencies bove 8 kHz (Fig 3) while the neomycin damge could affect all frequencies above 2 kHz Figs 4, 5)

A steep sloping threshold curve was oberved in several animals (A 19 10 kHz, 10 dB, 2 kHz, 25 dB, 16 kHz, 32 5 dB, 20 kHz, 5 dB 25 kHz, 70 dB, 31 5 kHz, 70 dB and 40 Hz, 42 5 dB) Some atoxyl treated animals in his group displayed differing degrees of hreshold shift in both ears of the same animal Fig 3)

The histopathological examination revealed one animal (Fig 6C) with a more extensive earing loss (A 10 1 kHz 25 dB, 2 kHz, 37 5 4 kHz, 50 dB, 8 kHz, 52 5 dB 16 kHz, 7 5 dB, 31 5 kHz, 72 5 dB) severely damaged in the whole cochlea (78–90%) while the IHC were considerably less affected (20– ?%) The three rows showed equal OHC

The neomycin treated animals showed an initial threshold shift as a high tone loss with e threshold ...

the lower frequencies There appeared a good correlation between the threshold of the two ears of the same animal Two animals displayed no threshold shift at frequencies 1–8 kHz whereafter the threshold decreased rapidly e g (N 15 8 kHz, 15 dB, 10 kHz, 30 dB, 12 kHz 45 dB, 16 kHz, 55 dB, 20 kHz, 60 dB, 25 kHz, 70 dB, 32 kHz, 65 dB, and 40 kHz, 45 dB) (Fig 7) The histopathological examination revealed extensive damage in the basal coil of the OHC which were totally lacking The IHC were considerably better preserved, showing a 30–40% loss in the basal part and less than 20% were degenerated in the apical part of the basal coil Unfortunately it was not possible to investigate the basal hook in these animals In the basal part of the 2nd coil 36% of the OHC and 3% of the IHC had suffered damage (Fig 7C) while the upper part of the coil showed a normal hair cell count This was also true concerning the apical coil except for the most apical mm of the cochlea which re vealed irregularities among both OHC and IHC

With increasing threshold shift (Fig 4) in the range between 4 and 8 kHz (N 11 5 kHz, 15 dB, 6 3 kHz, *30 dB*, 8 *kHz*, *45 dB*) there was an increased hair cell damage as revealed by microscopic examination In the basal coil ap- ...ly 80% IHC were missing and the

OHC had totally disappeared (Fig 8) In the basal part of the 2nd coil 55% of the OHC were missing and 12% of the IHC but the upper part of the same coil still appeared mainly intact The apical coil demonstrated 20% missing IHC and somewhat fewer OHC The hearing in frequencies 1–4 kHz was normal

Comparison of the threshold shift with the light microscopic interference contrast findings revealed in general a close correlation between the hair cell degeneration pattern and the threshold shift Animal N 14 (Fig 9A), showed an evenly descending threshold shift curve from 1 kHz to 6 3 kHz (1 kHz, 15 dB, 1 6 kHz, 25 dB, 2 kHz, 30 dB, 3 1 kHz, 40 dB, 4 kHz, 50 dB, 5 kHz, 55 dB, and 6 3 kHz, 60 dB) Above that frequency no measurable response was recorded The histological examination disclosed that well preserved hair cells occurred only in the upper part of the 2nd coil (Fig 9B) At apex both types of hair cells showed irregularities, though less than 10% were actually missing

iform threshold shift

imals recorded in this group had threshold ifts of 30–40 dB and often combinations with no physiological response within a part of the investigated frequencies This type of inner ear damage was found both in animals treated with atoxyl and neomycin (100 mg/kg b w on each occasion of both substances respectively with a total dose of 1 200 mg/kg of neomycin and 400 mg/kg of atoxyl) The threshold shift was rather similar in both groups often showing an impairment of 40–50 dB at 1–8 kHz (Figs 11, 12)

The time between the last injection and the sacrifice of the animal was of great importance following neomycin administration In animals having received the same total amount of neomycin and investigated 45 days following the last injection only 1 of 6 rats had a total threshold shift at frequences above 8 kHz compared with 6 of 6 in the group measured 102 days after the end of the treatment

The histological examination of the cochleae showed severe damage to the OHC w often were present only in the most apical of the cochleae The IHC were also seve affected in many parts of the cochlear c Scattered smaller groups of both OHC IHC could be found at several levels in cochleae though not in the basal coil In a profusely destroyed cochleae also the porting structures revealed significant da in many places, especially in the basal co entire structural organization of the org Corti had disappeared (Fig 10)

Deaf animals

Two animals were injected with 200 m day of neomycin with a total dose of 140 kg No response at all could be elicited investigated 7 respectively 26 days follo the last injection

DISCUSSION

A variety of ototraumatic agents are k and their effects on hearing are well mented (review Hawkins, 1976) In the of comparative otopathology Hawkins described how noise, drugs and aging ca duce indistinguishable patterns of co pathology with subsequent hearing in ment or even total loss

In the present investigation the first el physiological threshold changes in the ra peared in the high frequency range 20–4 following both atoxyl and neomyci ministration thus indicating a hair cell tion in the basal part of the cochlea Th in agreement with the results of the mo logical investigation where hair cell los various degrees were found in anim which the threshold shift was conside Initially, however, only a scattered m hair cell degeneration was observed i basal coil without any predominance w gard to OHC and IHC

In animals that displayed a minimal t old shift without distinct hair cell degene in a specific region of the cochlea this m

erpreted in two ways either the threshold ft is a result of a dysfunction of only the al metabolism of some hair cells while ir basal metabolism still continues suffi ntly or it is a result of an imbalance of the iole hair cell homeostasis though without ing fully degenerated Since the threshold measurement and the histopathological of the present investigation were made after the last administration of the drugs, latter hypothesis seems the least probable reversible effect (disturbance of the func- l metabolism) on hair cells following ycin administration has been reported Wersall & Flock (1964) Morphological and iological correlates on hair cells with im ured metabolism have been reported by Kar- ick et al (1976)

The location of the basilar membrane of oxyl induced hair cell degeneration in the lea thus appears to be species dependant nniko (1976) showed apical affection of OHC IHC as a result of atoxyl administration nea pig) while in the present study no se- ctive damage occurred among hair cells in apical coil of the rat cochlea On the con- ary the earliest and most frequent hair cell anges were found in the basal part of the rat In comparison, the neomycin- duced hair cell changes occur mainly in the asal coil both in the guinea pig (Stebbins et , 1969, Jauhiainen et al , 1972, Ylikoski, 74) and the rat, the latter confirmed by the esent study

In atoxyl treated animals with a sloping old shift discrepancies in the threshold hift sometimes appeared in both ears of the ame animal while the inner ear pathology of neomycin treated animals revealed a ather uniform picture in the two sides The ason for this pattern may be explained by a nism for a semipermeable system con ing the blood/endolymph barrier Stupp 1970) showed that the serum level of amino- side antibiotics must exceed a certain before an inflow to the endolymph oc s Similar results were obtained by Ritter et al (1971) It is thus likely that the neomycin concentrations in the blood following the in- jections were sufficient during a period of time to allow passage through such a barrier while an administration of 75 mg/kg of atoxyl was very close to the borderline level for inflow to the cochlea Variations among individual animals in kidney function and circulation of inner ear fluids may also influence the suscep- tibility to ototoxic substances

Following a penetration into the labyrinth, both atoxyl and neomycin are retained in the cochlea during a long time and have a delayed elimination from the inner ear (Stupp, 1970, Stupp et al , 1973, Anniko & Plantin, 1977) However, both atoxyl and neomycin are nephrotoxic (Anniko & Ljungquist, 1977, Hawkins 1970) which following a prolonged administration may cause an accumulation of the substances in the body and thereby excert ototoxic effects in the concentrations initially not affecting the hair cells of the labyrinth

No significant difference occurred among atoxyl treated animals receiving approx- imately the same total amount when investi gated 18 days to 3 months after the last injec- tion in contrast to the neomycin induced hair cell degeneration, which during the first 1–2 months progressed with the survival time Several investigators have focused attention on such a mechanism in the development and course of hair cell damage following the ad- ministration of aminoglycoside antibiotics (Ylikoski, 1974, Anniko & Wersall, 1977, un published observations)

A correlation between the pure tone audio- gram and the cochlear pathology has been well documented in the guinea pig (Ylikoski, 1974) the monkey (Stebbins et al , 1969), the cat (Schuknecht & Sutton, 1953, Elliott & McGee, 1965), in man (Bredberg 1968) and others, while the rat, often used in physiological ex- periments, lacks comparative documentation

The number of animals with an abrupt hear- ing loss showing a relatively distinct change in hearing sensitivity at a narrow frequency range in the present study, however, was too

small to allow a detailed analysis of the anatomical frequency scale in the rat. Roughly, this may be interpreted as follows: the lower half of the basal coil has an area of maximal stimulation at frequencies above 20 kHz, the upper half of the basal coil corresponds to 10–20 kHz, the lower half of the 2nd coil includes 2–8 kHz and the upper half of the same coil contains frequencies of maximal stimulation below 1.5–2 kHz. The importance of the apical region of the cochlea in hearing is, however, still not established.

Some biologically active substances may have a high order of organ specificity but this does not necessarily mean that the location of their greatest effect within the organ is similar in different species. In a complex system such as the inner ear species-dependant variations can occur, e g. as shown in the present study concerning atoxyl and neomycin. Experimental pathology may benefit from studies on the differences in the pharmacological action(s) on the inner ear in different species of various [illegible]totoxic substances and the result of such [illegible]ies may result in bringing insight into the [illegible]ction of the ear.

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ZUSAMMENFASSUNG

Die Pathologie des Innenohres zufolge der ototoxischen Verbindungen Atoxyl und Neomycin wurde in der Ratte vermittels physiologischer und morphologischer Methoden analysiert Trotz den Verschiedenheiten in ihrer Wirkungsart verursachten beide Substanzen in den Anfangsstadien ihrer ototoxischen Wirksamheit in der Ratte eine Deterioration im Horen von hoheren Tonen mit einem folgenden morphologischen Degenerationsmuster in den Haarzellen in den Basenteil der Cochlea Die ausseren Haarzellen waren bedeutend ofter angegriffen als die inneren Haarzellen zufolge der Anwendung von sowohl Atoxyl wie Neomycin

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A PROSPECTIVE STUDY OF GENTAMICIN OTOTOXICITY

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Abstract Twenty patients were included in a prospective otoneurological study performed to assess the ototoxicity in gentamicin therapy Gentamicin was administered intravenously, and the serum level was currently determined Audiographic and electronystagmographic studies were carried out at the institution and discontinuation of the treatment and again a few weeks later Ten patients exhibited ototoxic actions predominantly cochlear, 4 of the cases *being fully reversible Two patients developed* severe hearing loss, associated in one with bilateral extinction of vestibular function Low serum levels of gentamicin did not rule out the possibility of ototoxicity These *results urge the continuing of prospective studies and in*dicate that gentamicin should be used only as a link in the primary treatment of severe infection or in cases in which other, less toxic agents have failed

Side effects of drugs upon the 8th cranial nerve are generally due to salicylates, certain diuretics, and aminoglycosides Among the latter a few affect mainly the vestibular and others especially the cochlear system

In retrospective studies the ototoxicity of gentamicin has been found to disturb vestibular function in two thirds of the cases and cochlear function in one-third Half the patients with signs of hearing loss also exhibit vestibular symptoms (Jackson & Arcieri, 1971)

In animal experiments using electron microscopy Wersall et al (1969) demonstrated that the ototoxic action of gentamicin consists in degeneration of the vestibular and cochlear ciliated cells The damage to the ciliated cells in the cochlear is less marked and manifest *later than the vestibular changes In the organ* of Corti, degeneration of the ciliated cells starts in the basal turn, that of high frequency perception, and progresses towards the apex

The damage to the sensory cells is possibly dependent on the concentration of genta in the endolymph Hawkins (1973) ha phasized, moreover, that the ototoxic might be due to changes in the gluco tabolism of the ciliated cells or to char the composition of peri and endo *caused by the vascular changes* even gentamicin

It still remains unelucidated whether cular high concentration in the blood time after parenteral administration of micin (the peak concentration) is a fa the ototoxicity, or whether it is rather ration of an elevated concentration as by the gentamicin level in the blood i ately before the next dose (the valley c tration) which is decisive (Banck et al Hewitt, 1974, Noone et al, 1974 Noo et al, 1973) Possibly both factors are tive, *as it may be the area of the 24-ho* centration curve which ought to be rel possible signs of ototoxicity (*Lancet* Tjernstrom et al, 1973)

The incidence of gentamicin induce toxic damage has been inadequately dated, as the publications have so fa based upon retrospective studies

The present investigation was an atte assessing by a prospective study the inc of toxic damage by gentamicin to the 8th al nerve

PRESENT INVESTIGATIONS

Material and Method

The material comprises 20 patients (12 and 8 females) admitted to Medical Dep

entofte Hospital from 1 11 73 to 28 2 75 iese patients 17 were suffering from a nant, radical haematological disease (in ng 8 with acute leukaemia) and three from minated collagen disease None have on previous treatment with gentamicin none had prior otologic diseases The in ion for gentamicin therapy was severe in on which had usually arisen during inten cytostatic combination therapy During above mentioned period all patients were ided in the material, if their condition per d the fairly exacting examinations for a le of hours in the E N T Department in the first 48 hours of the antibiotic treat t The mean age was 49 years (range 16– years) Gentamicin was often combined i cephalothin and administered intrave sly in the course of 5 minutes The initial was calculated on the basis of body ght (80 mg for patients over 60 kg 60 mg those under 60 kg), while the subsequent es and the intervals were adapted also to serum creatinine Thereafter, the dose was d on the basis of the serum concentration entamicin which was measured for the first e on the 2nd–3rd day of the medication serum concentration of gentamicin was ermined by an Agar well diffusion test De ination of the concentration as well as of im creatinine were repeated at intervals of days done on samples drawn immediate before an injection (valley concentration) 45–60 minutes later (peak concentration) e aim was a peak concentration of 6–12 μg/ and/or a valley concentration of 1–3 μg/ml he mean duration of treatment was 8 days 16 days) and the total gentamicin dose d from 480 mg to 3 260 mg (mean) mg)

In the event of normal otoscopy the patients the following tests before or in the course the first 2 days of the treatment

a) pure tone audiometry and speech audiometric hearing test (T1),
b) vestibular testing by electronystagmography to detect spontaneous and position nystagmus as well as a caloric test stimulating by 44°C water for 30 sec by the method of Hinchcliffe (ENG) (2, 7)

These tests were repeated a few days after discontinuation of the medication (1st follow up) and again 3–19 weeks (mean 10 weeks) later (2nd follow up)

Four patients died between 1st and 2nd follow up

RESULTS

(a) Audiological

To assess cochlear function and to exclude the test–retest variation (Harris & Myers, 1954), we have defined the hearing loss at the individual frequencies as a reduction of the hearing threshold by more than 10 dB relative to the initial value The results are seen in Tables I and II

As might be expected, all hearing losses were most pronounced for the high frequencies

At 1st follow up, unilateral hearing loss was found in 3 out of 20 patients In one the hearing loss was present also at the 2nd follow up examination In another it was versible, while the third patient had unchanged hearing loss at 2nd follow up In the meantime however, this latter patient had been put on streptomycin, and there was also a suspicion of leukaemia involving the central nervous system, so that the result of the 2nd follow up cannot be taken into account No patient developed changes in T1

At the 2nd follow up 6 of 16 patients were found to have unilateral and one patient bilateral hearing loss in the range 15–30 dB (mean 20 dB) The serum gentamicin had been higher than intended in only 2 of these 7 patients In the only 2 patients who could be tested again one year later the hearing impairment proved to have been fully reversible Two of the 7 patients also developed a change in T1 of more than 10 dB, both had too high serum gentamicin It should be mentioned also that, before the 2nd follow up, 2 of the patients had re

Table I *Distribution of hearing loss in 3 patients at 1st follow-up and in 7 patients at 2nd follow up (one patient had a hearing loss at 2000 Hz as well as 4000 Hz at 1st follow up)*
Ti Threshold of intensity (speech reception threshold)

No of pats tested		Frequency (Hz)			
		2000	4000	8000	Ti
20	1st follow up	1	1	2	0
16	2nd follow up	1	1	5	2

ceived 2 and 3 courses respectively of gentamicin at intervals of a couple of weeks (total treatment period 11 and 16 days and total dose gentamicin 2640 and 3040 mg respectively)

(b) Vestibular

At 1st follow up 2 of the 20 tested patients had affected vestibular function One of them had bilateral canal paralysis which proved to be permanent This patient had had a massive elevation of the valley concentration of gentamicin in the serum (5 6 μg/ml) The other patient had unilateral canal paresis which regressed in 8 weeks In this case the gentamicin concentration had been within the intended range

At 2nd follow up, performed in 16 cases, bilateral canal paralysis was still present in the above mentioned patient Another patient had unilateral canal paresis which proved to have been reversible at repeated follow up one year later The serum gentamicin in this patient had been within the intended range

DISCUSSION

So far, the real incidence of gentamicin-induced ototoxic damage has been unknown, the analyses published hitherto have been retrospective This applies to the studies of Banck et al (1973) Noone et al (1974) Wersstrom et al (1973) and Tjernstrom et al (1973) who demonstrated by acoustic vestibular tests, ototoxicity—as a rule subclinical (asymptomatic)—in 20% of the treated patients Ototoxicity causing symptoms was recorded by Jackson & Arcieri (1971) in of a very large patient material in the USA during the period 1966–69 Due to more careful monitoring of the treatment this rate seems to have fallen since then to around 1% (Hewitt 1974) A similar rate of ototoxicity with symptoms has been reported by Federspil & ... burg (1970) Since the hearing loss affects primarily the highest frequencies it is not noticed by the patient who is also able to correct by reflex for mild canal paresis ... vestibular apparatus This explains the low rate of ototoxicity in the last mentioned series

The present prospective study demonstrated a very high degree of apparently gentamicin induced ototoxicity Almost half the patients (9/20) developed hearing loss in

Table II *Ototoxicity probably related to therapy with gentamicin in relation to age, high valley concentration (HCV) and the serum creatinine*

Symptom	No of pats	Mean age (year)	Mean HVC (μg/ml)	Serum creatinine mean (mg/ml)
Auditory only				
Transient	2	52 (36–68)	2 4 (1 6–3 2)	0 8 (0 7–0 9)
Irreversible	5[a]	38 (17–55)	1 8 (1 0–3 2)	1 0 (0 5–1 5)
Vestibular only				
Transient	1	79	2 4	1 6
Irreversible	0			
Auditory and vestibular				
Transient	1	17	1 0	0 7
Irreversible	1	75	5 6	1 0

[a] One patient also received streptomycin

-ges were fully reversible, the other 6 died
itively short time after the second follow-
n at least 2 of the patients the hearing
irment was appreciable, being demon
le by conventional as well as by speech
y Except in one patient, with co-
ing bilateral extinction of vestibular func-
the hearing damage was unilateral Three
e 20 patients tested exhibited vestibular
ity which was unilateral and fully revers-
in two Two patients developed signs of
lear and vestibular damage, fully rever-
in one

ius at worst, there is a possibility of per-
ent ototoxicity (as a rule sub-clinical) in 6
of 20 patients In most cases the toxicity
not demonstrable at the completion of
tment, not until the 2nd follow-up exami
on some weeks later The cause of this
incidence of hearing damage is possibly
poor general condition of some of the pa-
ts who, therefore, did not cooperate very
Judging by the case notes, however,
general condition—and the haemoglobin
l—did not seem poorer at the subsequent
minations than at institution of the treat-
it None of the patients showing ototoxic-
was on furosemide therapy, and only 2
the patients with ototoxicity developed
lightly elevated serum creatinine in the
rse of the treatment, this might have given
to non recorded major fluctuations in the
tamicin concentration Two of the patients
h ototoxicity were over 70 years of age
mean age in this group was 46 years, as
npared with 49 years in the group without
toxicity Only one of the patients com-
ned spontaneously of hearing impairment
d dizziness) This is in agreement with the
dies reported above in which 1–2% of the
ents developed subjective symptoms of
toxicity

It has been pointed out by Hewitt (1974)
it the valley concentration of gentamicin
the serum in particular, is of value in
venting toxicity A valley concentration ex-
ng 1 5–2 0 μg/ml is said to involve an in-
creased risk of damage (Banck et al, 1973, Hewitt, 1974, Nordstrom et al, 1973)

Among the present 10 patients with ototoxicity, 4 had valley concentrations exceeding 2 μg/ml (2 4, 3 2, 3 2, and 5 6 μg/ml respectively) The highest valley concentration (5 6 μg/ml) was observed in the patient with bilateral canal paresis and severe bilateral hearing loss Only one patient had an elevated peak concentration of gentamicin in the serum (13 5 μg/ml)—and incidentally also a slightly elevated valley concentration (3 2 μg/ml) This patient developed severe unilateral hearing loss after having been treated, with minor interruptions, for 16 days with gentamicin in a total dose of 3040 mg In the last 2 patients with a slightly raised valley concentration the ototoxicity proved fully reversible It must be mentioned that in 4 patients without demonstrable ototoxicity the valley concentration had also exceeded 2 μg/ml (2 5, 3 6, 3 6, and 6 6 μg/ml), but only 2 of these patients were seen at the second follow-up In other words, even a very low valley concentration (<2 μg/ml) does not exclude the possibility of ototoxicity Similarly, valley concentrations up to at least 3 6 μg/ml have been observed without subsequent otological damage There does not seem to be any question of major fluctuations in the serum level of gentamicin, leading to difficulty in regulating the dosage in the patients with ototoxicity in contradistinction to those without At least, there was no difference between the two groups in the frequency of dose adjustments

As regards the total dose of gentamicin and the duration of treatment, our material shows no difference between the group of patients with and that without demonstrable ototoxicity Thus, 10 patients with ototoxicity received a mean dose of 1559 mg gentamicin (480–3040 mg), and the treatment period had averaged 8 0 days (5–16 days), whereas the 10 patients without demonstrable ototoxicity received an average of 1762 mg gentamicin (960–3260 mg) in the course of a mean treatment period of 7 9 days (5–12 days)

CONCLUSION

The present material is fairly small and nearly all the patients had malignant haematological disease treated intensively with cytostatics prior to the infection Owing to our favourable experience of controlling infection with gentamicin, it was not justified to run a control group of similar patients from whom gentamicin was withheld

Since ototoxicity proved to be a more common complication of gentamicin therapy than was earlier assumed, our results prompt us to continue with prospective studies, including patients with other radical diseases and including also other aminoglycosides for toxicological comparison

As a rule, the demonstrated toxicity does not give rise to symptoms It generally affects the cochlea, is often unilateral and presumably fully reversible in most cases Ototoxicity was observed in several patients with a very low serum level of gentamicin (valley concentrations of less than 2 μg/ml) Accordingly, the alue of determining the serum level lies more in securing optimal treatment than in excluding a risk of ototoxicity

Gentamicin should still be used only as a link in treating severe infections and infections in which less toxic agents have proved ineffective The treatment should be as brief as possible

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ZUSAMMENFASSUNG

In einer prospektiven otoneurologischen Untersuchung welche 20 Patienten umfaßte wurde versucht die Ototoxizität von Gentamycin zu beurteilen Gentamycin wur de intravenos appliziert und dessen Serumkonzentration laufend bestimmt Bei Beginn unmittelbar nach Abschluß der Behandlung sowie einige Wochen spater wur de ein Audiogramm aufgenommen sowie Elektronystagmographie durchgefuhrt Bei 10 Patienten konnten ototoxische Schaden festgestellt werden welche überwiegend cochlear und in mindestens 4 Fällen [illegible] reversibel waren Zwei Patienten zogen sich [illegible] Horschaden zu in einem der Fälle sogar mit [illegible] Vestibularfunktion Selbst bei niedriger Gentam[illegible] [illegible]

[illegible]

darauf hin daß Gentamycin nur als ein Teil der [illegible] Behandlung schwerster Infektionen vorbehalten se[illegible] te, oder wo andere weniger toxische Medikamente als wirkungslos erwiesen haben

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FLUORESCENT STAINING OF HAIR CELLS WITH ETHIDIUM BROMIDE

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act The present study evaluated ethidium bromide leic acid specific fluorescing stain for cochlear apons Tissue exposed to acoustic stimulation did not t the loss of fluorescence in hair cells described in other fluorescing stains The ethidium bromide scence technique was however found to be useful tecting subtle damage in cell nuclei even before gross tural alterations in cochlear cytoarchitecture ap d The implications of the use of ethidium bromide ing for histologists are discussed

nges in fluorescence in hair cells follow- acoustic stimulation were reported by dstein (1973) and Guth et al (1974) Gold- n (1973) using the appearance of the fluo- ent end product (fluorescein) of the es- tic hydrolysis of the non fluorescent fluo- ein diacetate (FDA) as an indicator of en atic activity in living cochlear tissue, ob- 'ed progressively diminished fluorescence orochromasia) induced by varying degrees coustic stimulation Goldstein concluded since the rate of hydrolysis of the FDA r to fluorescein did not change after acous- stimulation, the loss of fluorescence was , due to changes in cell membrane per- bility Guth et al (1974) investigated the of the organ of Corti by terphenyl- cholinium 3 (TPHC 3), a fluorescent ivative of hemicholinium 3, an inhibitor of ylcholine synthesis (MacIntosh et al, 1958) Guth and his associates intended e TPHC-3 to map the efferent (cholin c) olivo-cochlear bundle (OCB) innerva of the organ of Corti The results of their nt on fixed cochlear tissue however, suggested that TPHC-3 was not taken up by efferent nerve endings but by what the authors surmised to be cell nuclei After exposing cochlear tissue to ototoxic or acoustic trauma, Guth et al observed a loss of fluorescence associated with hair cell nuclei Because it was felt that the TPHC-3 cation might be reacting with acidic moieties in cell nuclei such as nu cleic acids, it was decided to use ethidium bromide (ETHBr), a nucleic acid specific fluorescing stain (LePecq et al 1964, LePecq & Paoletti, 1967)

The present study was designed to evaluate ETHBr for cochlear applications Animals were subjected to acoustic trauma to determine whether ETHBr exhibited a stimulation-dependent reduction of fluorescence as described by Goldstein (1973) and Guth et al (1974) Under the conditions used in the pres ent experiment, no loss of fluorescence was observed in cochlear tissue after sound exposure but other valuable observations made in the course of this study are reported here

METHODS

Guinea pigs weighing between 200 and 500 grams with active Preyer reflexes were used Animals were sacrificed by decapitation and prepared for histological study using the fol lowing procedure Temporal bones were im

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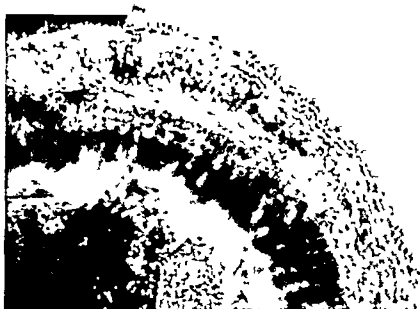


Fig 1 UV photo showing fluorescent produced by ETHB fixation Magnificati ×100

mediately removed and their bullae opened Tiny openings were made in the apical and basal ends of the cochleae These cochleae were then submerged in 2% OsO_4 solution for 10 min and gently perfused through the openings to assure adequate fixation Traces of sO_4 were removed in two physiological sa ne rinses The bony coverings of the cochleae were removed leaving the spiral ligaments intact These cochleae were then submerged in ETHBr solution (10 ³M) for 10 min Stained cochleae were dissected in 50% glycerol under a dissection microscope (Wild M-5) The basilar membrane and parts of the osseous spiral lamina were dissected from the cochleae and placed on glass slides The tissue was immediately examined under ultraviolet (UV), phase contrast, and simultaneous UV-phase contrast microscopy using a Wild M 20 micro scope

Animals receiving acoustic stimulation were exposed to 4 kHz tones at 110 db SPL lasting up to 90 min The stimulating signal was gen erated by an audiometer and was amplified by an Altec A340A amplifier Signals were pres ented to the animals through an Electro Voice SP12 speaker in an Industrial Acoustics Com pany type 1202A sound treated room Before stimulation test animals were anesthetized with an injection of sodium pentobarb mg/kg i p) and taped to a stage in front speaker The animals were prepared for logical study 24 hours after the termina acoustic stimulation

RESULTS

The fluorescent pattern produced by E (Fig 1) clearly indicates the affinity stain for the nuclei of the cells of the o Corti In Figure 1, the regular rows o rescing structures at the periphery (t right of Fig 1) of the basilar membra resent nuclei of outer hair cells (OH Deiters' cells The inner sulcus area seen to contain a number of nuclei of shapes The dark band in the center of is associated with the bone of the o spiral lamina The area of ETHBr fl cence in the bottom center of Fig 1 is ner s membrane which was folded bac the organ of Corti during dissection shows a detail of the ETHBr fluoresce the same area as depicted in Fig 1 The regularly spaced nuclei belonging to OH evident in this preparation The inco row of nuclei at the periphery of the (top of Fig 2) belong to Hensen s cells

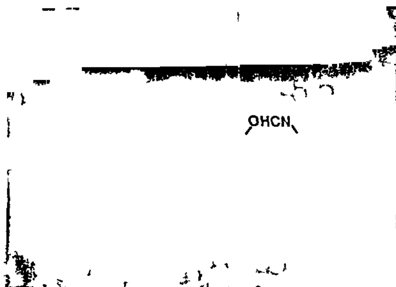


Fig 2 UV photomicrograph showing a detail of the ETHBr fluorescence in the same tissue as in Fig 1 OsO_4 fixation Magnification ×500 OHCN outer hair cell nuclei HC Hensen s cell nuclei *T* tunnel *IS* inner sulcus

the Hensen s cells and other cells of the er sulcus region as well as the spiral liga nts were removed during dissection The a below the OHC nuclei which is devoid fluorescence is the tunnel (*T*) Some of the clei of the inner sulcus (*IS*) belong to inner ir cells (*IHC*) but it is difficult to differen te these cells without the aid of phase ntrast microscopy An interesting band of THBr fluorescence extends inward (downward in Fig ?) from each of the first row OHC nuclei This fluorescence might be illumination of the hair cell bodies by the fluorescing nuclei In some preparations these fluorescent bands terminate in barely visible spots of fluorescence which correspond with the positions of cuticular plates of OHC

Fig 3*a* is a phase contrast photomicrograph of the nuclear region of a row of OHC The same cells photographed under UV light con

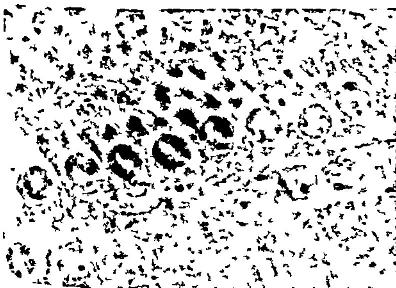


Fig 3a Phase-contrast photomicrograph of the nuclear region of a row of OHC OsO fixation Magnification ×1?00

78?953



Fig 3b UV p graph of the sa in Fig 3a ETI rescence OsO Magnification :



Fig 4a Phase-contrast photomicrograph of the reticular lamina OsO$_4$ Fixation Magnification ×1200 Arrow points to the cuticular plate of OHC referred to in Figures 4b and 4c

ditions (Fig 3b) clearly show the ETHI rescence in the nuclei The fluorescen tling which was a consistent finding in our preparations suggests that ETH staining the interior structures of the n It is not clear whether these hyperfluore structures are masses of chromatin materi nucleoli The row of nuclei which appea blurred fluorescent images at the bottom Fig 3b are Deiters cell nuclei which deeper in the tissue

ETHBr fluorescence in cells exposed to acoustic stimulation

The fluorescence produced by ETHBr in t sue exposed to high intensity acoustic stimul tion was carefully evaluated to determine i fluorescence was diminished No observab difference in brightness of ETHBr fluore cence was found between cochlear tissue which had been exposed to acoustic stimula tion and control tissue Even in cells which were in an advanced state of degeneration, ETHBr fluorescence was unchanged

A number of explanations for the lack of fluorescence loss are possible First improper stain concentration might have obscured a



4b Phase-contrast photomicrograph of the nuclear ion of a damaged OHC n the same area as in Fig OsO fixation Magnification ×1200

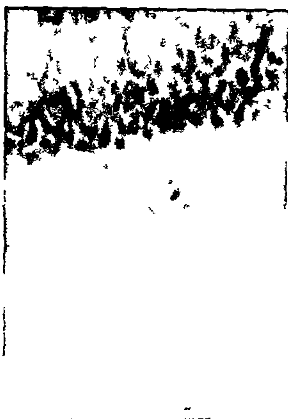


Fig 4c Simultaneous UV phase contrast photomicrograph of the nuclear region of the cell shown in Figure *4b* ETHBr fluorescence OsO_4 fixation Magnification ×1200

ise-induced changes in nucleic acids Secondly the 24 hours between the end of acoustic stimulation and staining of cochlear tissue may have prevented observation of any reversible changes in nucleic acids lasting less than 24 hours and any long term changes which would not have appeared at the time of sacrifice It is also possible that acoustically induced agitation of DNA and RNA helixes may increase the number of available binding sites and in fact increase ETHBr fluorescence as suggested in a study by Nicolini et al (1974) Several studies have indicated that changes in nucleic acid concentrations do occur in the organ of Corti after acoustic stimulation (Nakamura 1967 Kluyskens 1963 Vinnikov & Titova 1958 Ohhashi 1960 Kon 1964) It may be that two processes differing in sign (i e an increase in ETHBr binding and a decrease in nucleic acid concentration) cancel each other and cause no loss in fluorescence

Applications of ETHBr in cochlear research

The ETHBr fluorescence technique was found to be especially useful in detecting subtle damage in cell nuclei of tissue exposed to acoustic stimulation ETHBr fluorescence was in many cases able to detect such damage even before the familiar gross alterations in cells and the reticular lamina characteristic of acoustic trauma appeared

Because of its sensitivity the ETHBr technique appears to be an effective tool for damage assessment that has some advantages over the standard cytocochleographic technique a common histological procedure which ac



Fig 5a Phase-co tra photom crograph of t cular lam na 0 0 f Magnif cation ×1200

counts for the status of hair cells by the ap pearance of the reticular lamina The disad vantage in the use of cytocochleography can be seen in Fig 4 The OHC in this tissue would probably be classified as intact using the criteria of some cytocochleographic pro dures However examination of the sub ticular region reveals a misshapen hair cell ody even though the cuticular plate itself ap pears to be normal (Fig 4*b*) The same tissue examined with the ETHBr techn que (Fg shows a shrunken nucleus a clue to po pathology within this cell A similar s u is depicted in Fig 5 The cut cular plate n of this section of basilar membrane ap to be intact although some swell ng and tortion is suggested in the th rd row of plates A histological procedure wh ch d account for the subcuticular and nuclear ments of these cells m ght well have



Fig 5b Phase-con rast photom crograph of th clear region of OHC in same area as shown in 5a The arrow ind ca m ss ng nucleus 0 0 tion Magnif cation ×



Fig 5c Simultaneous UV phase-contrast photomicrograph of the nuclear region of cells shown Fig *5a* and *5b* ETHBr fluorescence OsO_4 fixation Magnification ×1200

oked the missing nucleus in an OHC which ppears to have an intact cuticular plate As en in Fig 5a–c this damage is clearly evi- nt with the aid of ETHBr fluorescence If gnificant numbers of such abnormal hair lls are overlooked in an examination of the ticular lamina the resulting high false nega- ve error might seriously compromise the ac- uracy of the histology and any conclusions erived from it

CONCLUSION

he present study employing UV and phase- ontrast microscopy has determined that the uorescing dots observed by Guth et al (1974) cochlear tissue stained with TPHC 3 were ell nuclei Photographic evidence presented the present study suggests that ETHBr uorescence was exclusively associated with ell nuclei in the organ of Corti and was re- arkably similar to the pattern of fluorescence uced by TPHC-3

Although acoustic stimulation did not pro- uce a loss in ETHBr fluorescence similar to at in studies with other stains (Goldstein, 1973 Guth et al , 1974), fluorescent staining of uclei in the organ of Corti proved to be a valuable histological tool Subtle forms of damage which were not readily visible in standard surface preparations of basilar membrane from sound treated animals were detected using the fluorescent staining technique

SUMMARY

Guinea pigs were subjected to acoustic stimulation at 110 dB SPL for up to 90 minutes to determine if the fluorescence of ethidium bromide a nucleic acid specific fluorescing stain, diminished in cochlear tissue The loss of fluorescence previously reported by Goldstein (1973) and Guth et al (1974) in studies with other stains, was not observed The use of ethidium bromide in surface preparations of basilar membrane was found to be especially useful in detecting subtle forms of noise-induced damage in the organ of Corti

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ZUSAMMENFASSUNG

Meerschweinchen wurden akustischen Stimulationen auf 110 dB SPL bis maximal 90 Minuten augsgesetzt, um zu bestimmen, ob die Fluoreszenz des Ethidium-Bromids eine nukleinsäure-spezifisch fluoreszierende Farbung im Schneckengewebe vermindert hat Der Verlust der Fluoreszenz, früher bei Goldstein (1973) und Guth und Mitarbeitern (1974) berichtet, wurde während Untersuchungen mit anderen Farbungen nicht beobachtet Die Benutzung von Ethidium Bromid an Oberflachenprapara ten der Basilarmembran wurde als besonders empfehlungswert fur den Nachweis von geringen Anderungen in der gerauschinduzierten Beschädigungen der Schnecke erachtet

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A STUDY OF THE VIBRATION OF THE BASILAR MEMBRANE IN HUMAN TEMPORAL BONE PREPARATIONS BY THE USE OF THE MOSSBAUER EFFECT

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tract Using the Mossbauer technique we have lied the vibration of the human basilar membrane and leus head in the sound frequency range 0 2–9 0 kHz at a sound pressure of 100 dB at the tympanic mem ne The displacement frequency response curves for basilar membrane and the malleus head have similar pes with a maximum at about 1 kHz Below and above Hz the curves have a slope of about 10 dB/octave and 00 dB/octave respectively In addition the basilar mbrane has a maximum displacement at a frequency endent distance from the stapes A simple hydro- namic model for the cochlea is used to interpret the erimental data A possible mechanism for the fre ency resolution of sound by the ear and noise induced aring loss is discussed

ne development of a satisfactory theory of aring depends on measurements of the dis acement characteristics of the basilar mem- ane in the cochlea Such measurements ere performed by von Bekésy (1960) during e period 1924 to 1958

Bekesy put the stapes into sinusoidal vibra- ons with constant amplitude and measured y the use of a microscope under stroboscopic lumination, the displacement as a function of quency at various points along the cochlear ition Using human ears (cadavers) and the from many species of animals, a series of urves relating relative amplitude to frequency obtained As a function of frequency the amplitude for each point on the resembled a resonance curve His nts on human temporal bone prepara were restricted to the frequency region low 2 kHz and to very high sound pressure vels (~140 dB re 2×10⁻⁵ Pa) Johnstone & Boyle (1967) applied the Mossbauer technique to measure the displacement amplitude of the stapes and the basilar membrane of living guinea pigs Rhode (1971) used the same tech- nique to measure the transfer ratio between the basilar membrane and the umbo of the mal leus in squirrel monkeys In both works the frequency response curves were found to have a positive slope (~10 dB/octave) on the low frequency side (below 1 kHz) and a rather steep negative slope (~−100 dB/octave) on the high frequency side (above 1 kHz) Kohl loffel (1972) introduced a new method to study the vibration of the basilar membrane This method is based on the fact that when illu- minated by a laser beam individual speckles on a surface become fuzzy when the surface vibrates with sufficient high frequency and amplitude With this technique he measured the frequency response curves in living guinea pigs and found similar values for the slope as those of Johnstone & Boyle and Rhode In dead specimens the values for the slope were found to decrease slightly with time after death

The use of a capacity probe to measure the basilar membrane vibrations was first sug- gested by Bekesy, but his probe was not small enough to give satisfactory spatial resolution Wilson (1973) using a sub-miniature probe with a tip diameter of 0 2 mm, was able to measure vibrations down to 0 1 nm

In the present work we have used the Moss- bauer technique to obtain values for the ampli

tude and phase for the head of the malleus and at various positions on the basilar membrane in human temporal bone preparations An attempt is made to fit to the experimental data values calculated by using a hydrodynamical model for the cochlea The implications of this model on the theory for hearing and on the loss of hearing shall be briefly discussed

EXPERIMENTAL

The experimental arrangement consists of the following four parts (i) an acoustical stimulus apparatus with which sound is applied to the external ear of a temporal bone, (ii) a Co 57 gamma (γ) source placed either on the basilar membrane or on the head of the malleus, (iii) an Fe-57 absorber (at rest) placed approximately 2 cm away from the source, and (iv) a γ detection system Parts (ii), (iii) and (iv) comprise a Mössbauer spectrometer The spectrometer, the acoustical stimulus apparatus and the preparation of the temporal bones are described in Sections A, B and C low

A *The Mössbauer spectrometer*

For the description of the Mossbauer effect on which this spectrometer is based we refer to a text book in nuclear physics The working principle of the spectrometer is as follows The source may emit and the absorber may absorb a 14 keV γ in a recoilless process When the absorber is placed between the source and a detector the counting rate as a function of the relative velocity between source and absorber then comprises the Mossbauer spectrum In our case both Co 57 and Fe-57 are embedded in a Rhodium metal foil in which case that spectrum will give one line of Lorentzian shape The full width at half maximum, Γ, of that line was measured for our system to be Γ=(0 360±0 005) mm/s

The sources were cut from a 1 mm×1 mm×4 μm Rhodium foil containing 30 mCi Co 57 In the measurements of the vibration of the basilar membrane and of the head of the malleus, the sources had an area of about 50 μm× 50 μm and of 200 μm×200 μm respectively The weights 0 2 μg and 3 μg of these sources are too small to significantly distort the movements of membrane and the malleus

The absorber is a 4 μm thick Rhodium foil with diameter 15 mm containing 5 atom Fe 57 This is an optimum level of enrichment in Fe 57 giving a maximum dip in the Mössbauer spectrum without broadening and distortion of the line shape

The detector is a scintillation counter (model MSP-1 from Elscint) with a 0 2 mm thick NaI(*Tl*) scintillator crystal shielded with 0 2 mm thick Be window which permits passage of low energy gamma rays The photomultiplier tube is an EMI type 9656 The counting efficiency for the 14 4 keV radiation is 100% and the resolution is approximately 40% when the tube is operated at a high voltage of 1 kV The pulses from the detector are fed to an amplifier with a single-channel analyser which discriminates pulse heights resulting from the lines of the source pulse height spectrum other than the 14 4 keV line

B *Acoustical stimulus apparatus*

To produce a sinusoidal stimulation a pure tone sine wave from an oscillator (Bruel & Kjæer, B & K Beat Frequency Oscillator type 1022) was used to drive an artificial voice (B & K, type 4215) The sound pressure was fed into the external auditory meatus through a plastic hose

Through a circular hole made in the meatal wall 2–3 mm from the ear drum, a microphone probe 4 mm in diam and 14 mm long was firmly cemented Using this probe the sound pressure level was measured with a B & K inch condenser microphone with total probe length of 20 mm With this configuration the output of the probe microphone at constant free field sound pressure level was constant within 0 5 dB for frequencies up to 1 kHz resonance occurred at 3 2 kHz This produced a sensitivity enhancement of 25 dB at the frequency To dampen this resonance peak

of steel wool was inserted into the 6 mm part of the probe nearest to the diaphragm he condenser microphone The condenser had a linear behaviour up to B SPL

Preparation of the temporal bones

Addition to the cementation of the micro- ne probe tube, described above, the prep ion consisted of making access to the per place on the basilar membrane The leus and part of the incus were exposed ough an opening in the tegmen tympani

Through this opening it was easy to observe movement of the head of the malleus under microscope with stroboscopic illumina- n It would have been preferable to use the placement of the footplate of the stapes as a rence, but this structure is far less acces- le for direct observation

With a dental drill the bone covering the la tympani was removed until a thin bony ll was left A small hole (~0 5 mm²) was ally made with a dissecting instrument The e was made at different places in the basal n of the cochlea where the characteristic ces for middle and high frequencies are ex- ted to lie The fluid of the scala tympani s removed and the small source was placed the basilar membrane with a glass needle most cases, when the scala tympani had en properly drained, the surface of the basi- membrane become quite sticky so that the urce attached firmly to the membrane If me fluid was left on the basilar membrane, e source would fasten to the surface mem ane of the fluid instead, thereby causing the urce to settle down improperly When this ppened, the source had to be removed and e process repeated After the source had properly placed, the scala was refilled, th cerebrospinal fluid, which is similar to e perilymph with respect to viscosity and rface tension This refilling did not affect the ient of the source to the basilar mem A pipette with a capillary tube tip was ed to refill the scala The tip had to be guided into the scala and the pipette pressed carefully in order to avoid the formation of air bubbles in the scala It proved to be exceedingly diffi cult to remove such bubbles The most suc- cessful method was to dip a small part of the bone into water and turn on an ultrasonic field for a short time This treatment, in most cases, caused the bubbles escape to the surface

The hole was then covered with a thin plas- tic sheet to keep the fluid level constant dur ing the experiments No hydraulic isolation was achieved with this plastic window

To protect the temporal bone from drying and thereby preventing changes in the proper ties of sound conduction, the laboratory was kept at a relative humidity of 60 to 70%

After the measurements of displacement and phase had been completed and the sources removed, the scala tympani was opened in order to measure more accurately the position of the source on the cochlear partition

RESULTS

A *Displacement amplitude*

For a harmonic oscillation the displacement amplitude y_0 at a sound pressure p at the tym panic membrane is given by

$$y_0=\frac{\Gamma}{2\pi f}\left(\frac{1}{(1-G)^2}-1\right)^{\frac{1}{2}} \quad (1)$$

Here, Γ is defined above, f is the frequency and

$$G=\frac{N(p)-N(0)}{N(\infty)-N(0)} \quad (2)$$

where $N(p)$, $N(0)$ and $N(\infty)$ respectively, are the counting rates measured at the pressure $p=100$ dB (re 2×10^{-5} Pa), with no stimulus and with the system heavily over stimulated ($p=130$ dB at 1 kHz)

Values for y_0 are given in dB relative to 0 1 nm To improve resolving power, the ac- tual sound pressure p was sometimes more than 100 dB The data were then extrapolated to $p=100$ dB, assuming the system to be linear

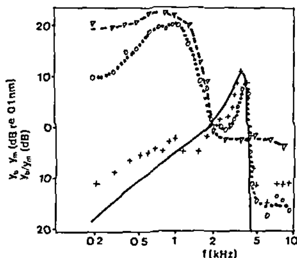


Fig 1 Frequency response of the displacement amplitude ∇ Malleus head (y_m) O basilar membrane (y_b) at $x = 12\ 0$ mm from stapes +, ratio y_b/y_m The solid curve represents calculated values for the ratio y_b/y_s where y_s is the displacement amplitude of stapes

Experiments showed that in the actual pressure range the system was indeed linear and hence the normalization did not introduce significant errors

The displacement amplitudes y_m and y_b for the head of the malleus and the basilar membrane, respectively, were measured in the frequency range 0 2–9 kHz for 7 points on the basilar membrane between (9 9±0 1) mm and (17 0±0 1) mm from the stapes For each source position, the response curves have similar shapes with a slight positive slope (about 10 dB/octave) below about 1 kHz and a steep negative slope (about 100 dB/octave) above that value In addition to the broad peak at 1 kHz the curves for the membrane exhibit a resonance peak, the position of which depends on the value of x and is characteristic for the membrane itself

A typical example is shown in Fig 1 with x at 12 0 mm The curves through y_m and y_b are drawn by eye In the same figure we have also plotted values for the transfer ratio y_b/y_m The corresponding curve represents calculated values for the ratio y_b/y_s where y_s is the displacement amplitude for the stapes In the calculation we used a hydrodynamic model for the cochlea, as described in the last section

Assuming y_s to be proportional to y_m we h[…] normalized the theoretical curve to the e[…]perimental values at the peak We see th[…] the calculated values reproduce quite well th[…] position of the membrane resonance pe[…] Above that peak the experimental data a[…]proaches a constant value, whereas the theo[…]retical curve approaches zero quite rapid[…] This discrepancy is probably due to slow le[…]age or evaporation of the liquid in the sca[…] The mass between source and detector w[…] then decrease causing the transmission of t[…] 14 keV γ to increase with time Since the r[…]erence rates $N(0)$ and $N(\infty)$ were measured […] the start of the experiment the value of […] rate $N(p)$ and thus of the displacement am[…]tude y_0 will increase with time Independ[…] experiments in which $N(0)$ was measured […]fore and after the measurements of $N(p)$ in[…]cated the presence of such a systematic er[…] This error is only serious at the highest […]quencies and varies from sample to sample […] the example shown in Fig 1 the error as re[…]sented by the tail of the membrane resp[…] curve is only about 0 02 nm Sometimes […] tail was as high as 0 1 nm

The measured values for the displacem[…] amplitudes varied from one preparatio[…] another

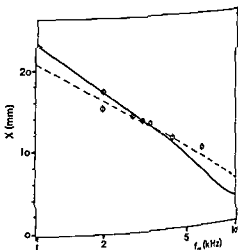
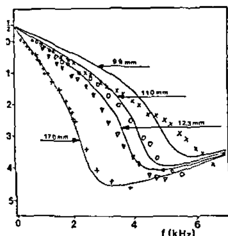


Fig 2 Relation between the frequency f_m and the pos[…] x on the basilar membrane of maximum displace[…] amplitude — Calculated values using our mode[…] Greenwood s formula (eq 3)



3 Phase frequency response at 4 different positions x the basilar membrane The points are experimental tes for the phase difference between the basilar mem ne and the head of the malleus The curves represent culated values for the phase difference between the nbrane and the stapes Values for the position x are n in the figure

A variation in sound transmission, measng volume amplitudes at the round window, is also found by Elpern & Andersen (1965) d Gundersen (1971) Gundersen (1971) und in 32 preparations that the patient's age death was without significance for the sound insmission the lapse of time between death id recordings being insignificant within wide nits Most people have experienced acute itis in the middle ear at least once Small echanical loss on account of adhesion after ich infections may be the cause of the obrved variations of the displacement ampli ides

We were not able to investigate the possible fect of the size of the opening of the scala mpani To do this properly, measurements n various sizes ought to have been performed the same specimen, which was not possible ue to the time factor

The calibration of the probe microphone another experimental problem he calibration procedure was carried out in a sound field at 100 dB SPL In the sound rements on the external meatus he probe tip was placed approximately 3 mm the drum This distance represents approximately 10% of the pressure wavelength at the highest frequencies or approximately an error of 0 5 dB in the pressure at 5 kHz and 2 dB at 10 kHz No correction for these errors was applied

In addition to the opening, the source itself might represent a disturbance to the movement of the basilar membrane The mass of the source is approximately 0 1 μg which is much less than that of the element of the cochlear structure upon which the source rests If the source is in good contact with the surface of the membrane, that is, if the stiffness of the coupling between membrane and source is high, the source will follow the membrane movement at high frequencies The only evidence that this coupling was strong is that the source was always found to be in the same place after the measurements had been com pleted

Due to these errors (discussed above) we believe that the experimental values for the transfer ratio can only locate the characteristic frequency, f_m, at which the resonance curve has its maximum In Fig 2 we have plotted on a semilog scale the position x of the source as a function of f_m The solid curve represents cal culated values using the hydrodynamic model and the broken line represents the following semi empirical formula according to Greenwood (1961)

$$x=35-16\ 7\ \log_{10}(0\ 006046 f_m+1) \qquad (3)$$

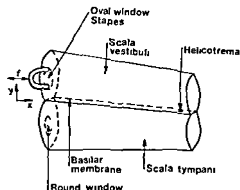


Fig 4 The uncoiled simplified model of the cochlea used in the calculations

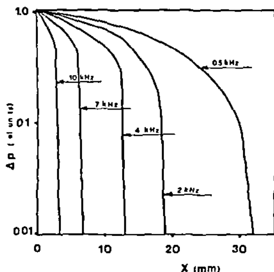


Fig 5 Calculated values for the pressure difference Δp across the basilar membrane as a function of the distance x from stapes at various frequencies

B *Phase response*

Phase response measurements were carned out in the following way Each time the signal from the condenser microphone passed through zero (on positive going) a trigger signal et the multiscaler to channel one Clock pulses with calibrated repetition time advanced the channel address within the period of the sound stimulus, giving a histogram with transmitted counting rate as a function of phase In Fig 3 are shown experimental results for the phase difference ϕ between the basilar membrane and the head of the malleus for four different positions along the membrane As seen from Fig 3 all the phase data start at $\phi=+\pi/2$ This is based on the assumptions that at low frequencies (i) the displacement of the membrane must lead that of the stapes by $\pi/2$ (Rhode 1971) and (ii) the head of the hammer and the stapes are in phase The curve drawn in the figure is theoretical and will be discussed in the next Section

The uncertainties in the experimental values of the phase of the basilar membrane relative to the phase of the middle ear structures are smaller than those of the displacement, hence the phase measurements are more reliable

DISCUSSION

A *Independent resonator hydrodynam... model*

The experimental data were found to be re... produced quite well by calculated values ... a model for the cochlea as suggested by Z... locki (1953) Typical examples of the ... already been given in Figs 1 2 and 3 We ... in the following only give a qualitative desc... tion of this model For a more comple... count, we refer to Zwislocki (1953) and S... stein (1976)

The model is based on the following ... sumptions

1) The coiling of the cochlea is unimport...
2) The fluid in the cochlea is ideal and in... pressible
3) The walls of the cochlea are rigid an... troduce resistance to the flow
4) At a position x on the membrane the ... cross section of the scala tympani is ... to that of the scala vestibuli and decr... slowly with increasing x
5) The membrane is a passive element a... regarded as consisting of a set of indep... ent resonators with no mechanical co... and having displacements which are ... relative to the cross dimension of ... scalas

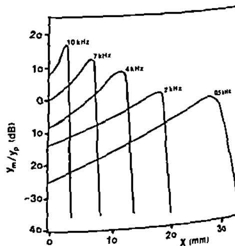


Fig 6 Calculated values for the ratio y_m/y_p betw... displacement of the basilar membrane and of the ... as function of the distance x from stapes at vario... quencies

hematic drawing of the cochlea is given in 4

sing this model we have calculated the sure drop Δp across the membrane and ratio y_m/y_s between the displacement litude of the membrane and the stapes as a tion of x at selected frequencies The re- s are shown in Figs 5 and 6 We see from 5 that at constant frequency Δp de ises with increasing x Furthermore, as the quency decreases, the length of the mem- ne which is exposed to a pressure dif- nce increases The curves for the displace- nt ratio given in Fig 6 have shapes typical resonance curves

qualitative description of the oscillations he cochlea is as follows The vibrations of footplate of the stapes at the oval window transmitted in the liquid in the scala ves uli and the scala tympani to the round win w At extremely low frequencies the whole ss of the liquid in the scalas participates in movement resulting in a pressure drop ng the whole length of the membrane As frequency increases the mass of the parti ating liquid and hence the length of the mbrane which oscillates decreases In ad ion to the mass, the resistance of the liquid gether with the elastic properties of the mbrane and the structure of the inner ear luences the position of the displacement aximum and oscillation characteristic of the mbrane

The results of the calculations as given in gs 5 and 6 shall now be discussed in relation the audio frequency resolving power and aring loss

Frequency resolving power

he tectorial membrane and the basilar mem ane are anchored at their inner edges at atially separate points, thus producing a earing force on the hairs when the basilar embrane vibrates The firing level of a nerve probably set by a lower level for the dis lacement amplitude It is interesting to note that according to our results at an input sound pressure level of 100 dB the maximum dis placement is of the order of 100 nm Assuming a linear relationship between displacement and sound pressure the displacement at the thresh- old of hearing is of the order of 10^{-3} nm which is far less than the thickness of a cell mem brane (~10 nm)

Of course such a straight extrapolation is not necessarily correct However, this indi cates that the firing level amplitude is so small that the basic mechanism in hearing is mole cular in nature The combined response of the middle and inner ear as measured for points of the basilar membrane between 9 9 mm and 17 0 mm shows that the displacement ampli- tude at frequencies around 1 kHz exceeds the displacement amplitude at the frequency cor responding to the maximum ratio between basilar membrane and head of the malleus dis placement The displacement ratio increases slowly with x to a maximum value, above which it falls rapidly to zero For a sound con taining several well separated frequencies the composite displacement ratio curve will con tain as many peaks and steep portions as there are frequencies The positions of the steep portions are much better defined than those of the peaks Hence it is more likely that it is the spatial derivative of the displacement ampli tude and not the amplitude itself that deter mines the firing level of the nerve

C *Hearing loss*

The results given in Figs 5 and 6 might ex plain why noise induced hearing losses appear first in the higher frequency region almost re gardless of the frequency components of the noise We shall illustrate this with an example Suppose the noise has a frequency of 0 5 kHz According to Fig 6 and the discussion above, the ability to resolve such a frequency depends on the nerves on the membrane in the region around $x \approx 30$ mm As seen in Fig 5 the largest pressure differences (and thus displacement) are, however, at the lowest values of x

Hence, damage to cochlea is most likely to occur in the region of the basilar membrane where the higher frequencies are resolved.

ZUSAMMENFASSUNG

Mittels der Mößbauer-Technik haben wir die Bewegungen in der humanen Basilarmembrane und in dem Caput Mallei studiert *Lautdruck ist von 100 dB im Frequenz-*gebiet 0,2 kHz bis 9 kHz angewandt Die Kurven der Bewegungsamplituden in der Basilarmembrane und dem Caput Mallei haben ein gleiches Aussehen mit Maximum etwa *1 kHz Unterhalb* und oberhalb *1 kHz fallen* die Kurven mit respektiven 10 dB pr. oktav und 100 dB pr oktav. Außerdem hat der Ausschlag der Basilarmembrane eine maximale Bewegungsamplitude abhangig von der Frequenz und dem Abstand des Stapes Ein einfaches hydrodynamisches Modell fur Cochlea is angewandt, um die experimentellen Data auszulegen Eine mogliche Erklarung, warum wir Larmschaden in besonderem auf speziellen Frequenzen bekommen, ist diskutiert

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DIAGNOSIS AND SURGERY OF ACOUSTIC TUMOURS

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ract In the diagnosis of acoustic neuromata the -dial reflex test the forward vs reverse Békésy test oudness balance test and the adaptation test have all ı found superior among the special tests used in pre tumour diagnosis Conventional X ray studies of internal acoustic meati are always performed fol by tomograms and in suspected cases by meatocis -ography In the present series translabyrinthine total oval was performed in 21 cases a planned decom sion in 5 while in 2 the removal was subtotal Only permanent facial paralyses arose one corrected by ssetenc plasty and the other by facial hypoglossal stomosis Otologists are urged to regard every uni ral perceptive hearing impairment as being caused by a our until proven otherwise

ologists have in recent years urgently felt ' need for early recognition of acoustic neu nata Earlier, this diagnosis was not con ered seriously until associated symptoms re present, e g reduction of corneal sensi- ity or atactic symptoms, in addition to im- ired hearing and vertigo The change of atti- de is due to the work of William House and ı associates (1964, 1968) who persisted in rfecting the technique of translabyrinthine mour removal until the results convinced NT surgeons all over the world that for small d medium sized tumours this method is un iestionably superior to the neurosurgical ap- oach

MATERIAL AND METHODS

his report is based on a material comprising 2 cases of acoustic neuroma and 36 cases of enière s disease to allow comparison of au- ological data In addition 29 cases are in- cluded in which a tumour was suspected but positive contrast meatocisternography showed normal findings The ages of the patients ranged from 21 to 76 years (mean 47 years) in the acoustic neuroma group, from 23 to 63 years (mean 40 years) in the Menière group, and from 11 to 63 years (mean 43 years) in the group of sensorineural loss with retrocochlear characteristics For preoperative audiologic evaluation, hearing tests for pure tone and speech audiometry, stapedius muscle reflex threshold measurements, Fowler's loudness balance and interrupted vs continuous tone Bekesy tracings as well as forward-reverse continuous tone tracings were carried out Possible pathological threshold was studied using the 3 min fixed frequency continuous tone stimulation

In the vestibular evaluation, tests for spontaneous and lateral gaze spontaneous nystag mus were performed and the caloric responses were recorded using water stimulation at both 44° and 30°C

In roentgen examination projection through the orbit, the Stenvers projection and skull base views were obtained Whenever indicated these were supplemented by tomographic examination of the internal meati, by positive contrast meatocisternography using 1 ml of contrast medium (Myodil) and 50 mg of cortisone, suspended in 1 ml spinal fluid In certain cases, finally, pneumoencephalography was carried out Surgical exploration of the internal acoustic meatus was performed in 28 cases of acoustic neuroma and in all 36 patients with Menière's disease

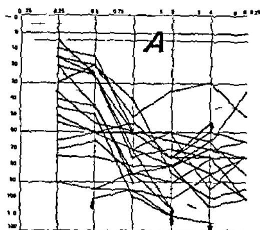


Fig 1 Individual air conduction curves in 23 neuroma cases (*A*) in 36 ears with *Meniere s disease* (*B*) and in 29 patients with sensorineural hearing loss with retro[cochl]ear characteristics but normal meatocisternogra[phy]

RESULTS

Preoperative evaluation

Shape of audiogram Fig 1 shows the airconduction audiograms of 23 cases of verified *acoustic neuroma* (9 were preoperatively *deaf*) The air-conduction curves for the group of 36 patients with Meniere's disease are presented in Fig 2 and those of 29 patients with audiological impressions of retrocochlear lesion but with normal filling of the meatus in meatocisternography in Fig 3 The general pattern in the tumour cases was downward sloping air-conduction curve, while the Meniere patients had flatter curves and the third group a mixture of both

The results of the various audiological tests included in our test battery are summarized in Table II Totally deaf ears when testing by air conduction appeared in all groups but the number of ears also deaf for speech was significantly higher in the neuroma group than in the two other groups Negative loudness recruitment appeared only seldom in Meniere's disease (27%) but significantly more often in the neuroma group In this series adaptation exceeding 30 dB at 500 Hz did not occur in Meniere cases, whereas in tumour cases it was observed in 26% and in the group of sensorineural deafness in 25% Adaptation at 2000 Hz brought the figure to 20% even in [the] Meniere group

Stapedius reflex threshold test was sig[nifi]cantly more often negative (70%) in the [neu]roma cases than in Meniere s disease (...) and the cases grouped sensorineural (2...) Conventional Bekesy testing did not s[how] significant differences between the gr[oups] whereas the forward vs reverse techn[ique] gave significantly more positive data in [neu]roma cases (63%) than in Meniere s d[isease] (4%)

The probability of arriving at the co[rrect] diagnosis by the various tests employed [in the] test battery was next evaluated The va[lidity] of each test was measured on the basis of [the] accuracy with which the test result had [pre]dicted neuroma in the cases where tu[mour] suspicion was verified The outcome is g[iven] in Table III The tests are arranged in o[rder] of decreasing validity and in this series the [sta]pedius reflex test together with the forw[ard vs] reverse Bekesy technique, emerged as [the] ones that most often accurately predicted neuroma diagnosis

Positive contrast meatocisternography [Ta]ble IV gives the results of the contrast stu[dy] divided into groups on the basis of the e[xamina]tion of the internal meati on tomogra[phy] Among 24 cases with normal roentgen [...]





2 Normal meatoc sternography X rays of left ear n through the orb t (*left*) and axially (*right*) Arrows it to contrast med um in meatus

s 3 tumours were found on 17 examina ns by positive contrast meatocisternogra y on the one hand while on the other nd out of 16 contrast studies with patho- gical plain roentgenographs 15 were posi ve for tumour

Fig 2 shows our normal roentgenographs after filling the meatus the X ray having been directed through the orbit through the maxil lary antrum and axially Examples of various abnormal results are seen in Figures 3 and 4

Pneumoencephalography was performed on





Fig 3 Absent fill ng but contrast medium droplets (ar- near the cochlear wall (*left*) R ght a tumour ex tends 1 cm into the angle Arrow po nts to the med al front of contrast med um around the tumour

6-789 3

Table I *Audiologic findings in three different disease groups*

Audiologic findings	Neurinoma (*N*=32)		Meniere s disease (*N*=36)		Sensori neural X ray normal (*N*=29)	
	N	%	*N*	%	*N*	%
Flat audiogram	11	34	32	89	21	72
Deaf ear, air conduction	10	31	8	22	2	7
Deaf ear, speech	17	53	10	29	8	28
Discrimination <60%	23	72	21	62	11	38
No recruitment (Fowler)	13	65	7	27	11	38
Pathological adaptation						
500 Hz	5	26	0	0	7	25
2 000 Hz	14	63	5	20	12	43
Stapedius reflex threshold >120 dB	14	70	6	26	6	25
Békésy Type III or IV positive	3	15	6	23	7	26
Forward vs reverse tracing positive	12	63	1	4	9	31

3 patients as the first procedure and as the findings were positive in all, meatocisternography was not undertaken

Complications of meatocisternography were seen in 3 of the 68 cases and included a high fever for 4 to 5 days, a stiff neck but no indications of a bacterial contamination All eactions subsided without sequelae and were, our opinion, caused by some oversensitivity the contrast medium on the part of the atient Symptoms of the cauda equina area irritation did not appear

Cerebrospinal fluid (CSF) protein

Table V gives the results of total determination in the three separate groups Only the values exceeding 100 mg/% seem to be concentrated in the tumour group but the majority of neuroma cases show quite low protein values

Treatment of neuroma cases

The established method of treatment is tu removal whenever possible Our arrange is to remove all small and medium size mours in the ENT Department If in d we obtain a pneumoencephalogram and tumour is found to leave a positive impre on the pons, the case is remitted to the ne surgeon If, on the other hand because patient's poor general condition or age neurosurgeon does not consider the neur gical approach feasible, we perform a ough decompression in the ENT Depart without attempting total removal

The surgical technique includes the labyrinthine approach with total mastoid my, desceletonization of the internal m and removal of bone from the sigmoid sin the cerebellopontine cistern Tumour is then removed from the meatus and the

Table II *Results of diagnostic tests*

Test results	Neuroma cases (*N* 32)		Meniere cases (*N*=36)		Normal X ray (*N*=29)	
	N	%	*N*	%	*N*	%
Deaf ears	10	31	8	22	2	7
All tests						
retrocochlear lesion	3	15	0	0	1	3
cochlear lesion	2	10	3	11	7	24




g 4 Filling defect produced by a 2 cm tumour (*left*) d by a 3 cm tumour (*right*) Arrows point to the medial ont of contrast medium around the tumour

rve identified in its peripheral end Intracapular removal is continued towards the pons nd by working from different directions, the imour capsule is gradually removed together with the tumour The connecting veins to the apsule are coagulated as they appear, and ius bleeding vessels are avoided in advance The cerebellum and the pons are protected y cottonoids which are inserted around the umour as soon as the intracapsular removal nas been adequate to allow shifting the tumour margins to the centre of the operating field In cases of large tumours, great care has to be exercised in separating the tumour from the IX–XI cranial nerve group, from the V nerve, from the anterior inferior cerebellar artery and from the pons Once the central part of the facial nerve has also been identified, the rest of the tumour can gradually be separated from the whole nerve With increasing experience even unexpectedly large tumours can safely be removed via the translabyrinthine route

Table III *Neuroma diagnosis Accuracy of audiological tests*

Test	Per cent accurate
Stapedius reflex >120 dB	71
Forward vs reverse tracing positive	71
Pathological adaptation >30 dB	
at 2 000 Hz	66
at 500 Hz	54
Loudness balance test	63
Speech discrimination >60 %	60
Békésy type III or IV positive	54

Table IV *Evaluation of meatal tomography in cases suspected to have acoustic neuroma related to results of cisternography*

Evaluation of meatal tomography		Cisternography	
		Negative	Positive verified by surgery
Normal	24	14	3
Questionable	22	18	2
Suspicion	15	12	3
Pathological	17	1	15
Total	78	45	23

Table V *CSF protein in three different disease groups*

CSF protein (mg/%)	Meniere s disease	Sensori neural hearing loss	Acoustic neuroma	
<50	8	23	9	
50–100	2	16	4	
>100		2	8	
Total number of patients studied	10	41	21	72

We remove the tumour tissue under slight hypotension and allow the blood pressure to return to normal when removal is completed All possible bleeders are cauterized by applying bipolar cautery, or weak current unipolar cautery The operative mastoid cavity is filled with fat taken from the abdominal area If the tumour has been small and the cerebellar dura kept nearly intact, no fixing of the fat is required If the dura has been split up to the moid sinus, the innermost large piece of fat fixed with catgut suture to the dural margins

Table VI shows the results during the years 1974 to 1976 Total removal was successful in 21 cases A permanent facial paralysis occurred in one case, later treated with masseteric transposition In 8 cases there was a temporary facial paralysis lasting from 2 weeks to 6 months and in 19 patients the facial nerve function remained intact the whole time In 2 cases the tumour could not be safely removed by the translabyrinthine approach and a second stage was done in the Neurosurgical Department One of them lost the facial nerve function completely but regained it in a very satisfactory manner after our hypoglossal facial anastomosis 3 months after tumour removal

Decompression was performed in 5 cases with total removal of the tumour from the internal acoustic meatus, and a large part of the intracapsular tumour In 4 patients a dramatic improvement occurred and they have been quite capable of taking care of themselves the fifth, a man of 76 years surgery as went without complications and the pa awakened normally but developed an pontine hemorrhage 4 hours later and d within a week without regaining conscio ness

All 21 patients with total removal in t ENT Department regained normal work capacity within 2 to 3 months

During the period 1973 to 1975 surgery performed on 44 patients in the Neurosurg Department The majority of these were la tumours causing a number of symptoms fr associated structures During the immed post surgical period the mortality was 3 an home 2 (11 %) The anatomic continuity of facial nerve could be preserved in 18 but t 8 (18 %) regained the function Fifteen pa could return to work after surgery (Tro 1977)

COMMENT

Unfortunately, audiological diagnosis of acoustic neuroma remains difficult and at p ent there are no tests not even a test batt that can give a definite confirmation (m mum 90 %) This, in addition to the rela rarity of these tumours, causes a delay in d nosis, which may be very unfortunate from operative point of view, the tumour gro into dimensions threatening the vital centr removal

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Operated cases	28
planned decompression	5
subtotal removal	2
total removal	21
Facial paralysis	
permanent	1
temporary	8
intact function	19

diagnosis or exclusion of the tumour, if re is a slight unilateral perceptive impairnt of hearing often combined with tinnitus d a somewhat lowered excitability in vesti lar testing At this stage we have the inner oustic meati X-rayed and apply part of the st battery referred to above On the basis of e data obtained the pure tone and speech sts are supplemented first with the stapedius flex threshold test and then with forward reverse Bekesy test (Palva & Jauhiainen 76) If these show recruitment and overlapng curves and the meatal X rays are normal, provisional diagnosis of inner ear deafness is ade and possible treatment initiated When ere is some X ray suspicion of meatal en-rgement or loss of bone density, binaural oudness balance and 3 min threshold adapta on tests are performed The routine Békesy iterrupted continuous tone tracing has in our xperience given less information and has late- / been omitted from the test battery as have ie SISI tests Stapedius reflex decay tests Anderson et al , 1970) have been conducted n some cases where reflex threshold could be ecorded In all three groups both normal and pathological decay could be observed Unfortunately the threshold for stapedius reflex was igher than 120 dB and no reflex decay could e measured in many cases of acoustic neu oma On account of great work load in the udiological department some test selection has become more and more pressing

Every case with unilateral perception deafness is followed once or twice a year and f the hearing loss or vestibular excitability shows further change, studies aimed at tumour exclusion are intensified The same applies to cases with continuously deteriorating bilateral perceptive impairments

As soon as it seems warranted meatocisternography with positive contrast medium is undertaken In our series of 68 examinations the side effects have been few and none seri ous, and no signs of radiculitis in the cauda area have appeared In this respect our results are similar to those reported by House and the risk of complications caused by the contrast medium seems to be minimal with the present preparations

Interpretation of the positive contrast meatocisternography always is easy if the tumour has already filled the inner meatus, or extended into the cerebellopontine cistern Difficulties have been encountered in the evalua tion of partially filled meati where part of the canal remains empty of the bulk of the con trast medium However, if small droplets are seen at the bottom of the meatus at the entrance of the nerves to the cochlea, we have interpreted the meatus to be free of tumour and in 4 such cases where vestibular and coch lear nerve neurectomies were performed there was no tumour but an increased amount of arachnoid mesh Nevertheless, if no surgery is indicated, careful following of the patient should continue and if objective signs in crease, a new cisternography can be undertaken later

Surgery of these tumours is always to be regarded as a major intervention and where and by whom it should be made depends upon the local situation In small countries like Finland with a population of 5 million, about 30 to 40 cases should be diagnosed each year This small number necessitates regional con centration as otherwise no one will have suf ficient experience in this surgery In Finland 90% of the cases are operated on in Helsinki, large tumours by agreement in the Department of Neurosurgery and the small and medium sized tumours in the ENT Department Close collaboration is essential in the selection and evaluation of the patients and should be done in the best interest of the patient

In ENT surgery the translabyrinthine approach affords excellent visibility for small tumour removal but careful anatomic dissection with application of bipolar cautery makes possible total removal even of large tumours However, in our experience the large opening to the field practised by the neurosurgeon adds to the safety of the procedure. The surgical technique as such as well as the possible com

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Acta Otolaryngol 86 241–247, 1978

PULSE VOLUME RECORDINGS IN OUTER EAR CANAL IN PULSE SYNCHRONOUS TINNITUS

A Comparison between Ears with Glomus Tumour, Serous Otitis Media and Normal Ears

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(Received August 17 1977)

Abstract With the aid of a volume flowmeter it is possible to record pulse synchronous volumetric changes in the outer ear canal In 7 ears with glomus tumour in the tympanic cavity and in 5 with serous otitis media such changes were larger than in 125 persons with a normal middle ear By changing the ambient pressure in a pressure chamber and instructing the patients not to swallow the drum can be pushed inward or outward In all the cases of glomus tumour studied the pulse volumetric change was considerably affected when the drum was pushed inward or outward In the normal patients the change was only small and in the 5 cases of serous otitis media there was no change at all This means that the pulse volume changes in normals are generated mainly by the vessels in the outer ear canal

This paper reports on the first of a series of investigations into tinnitus of middle ear origin Pulse-synchronous tinnitus is mostly caused by conditions outside the middle ear cavity, i e arterial hypertension or vascular anomalies but can also emanate from a glomus tumour in the tympanic cavity Visible tumour behind an intact ear drum, conductive hearing loss and pulse synchronous tinnitus are the three most usual manifestations of this disease (Spector et al , 1975) A sign easy to demonstrate and sometimes diagnostically useful is that described by Brown in 1953 i e visible diminishing and disappearance of the pulsations and paling of the area affected by the tumour on inspection through an airtight auriscope under increasing pressure in the outer ear canal

Ingelstedt et al (1967) studied middle ear mechanics with a flowmeter connected airtight to the outer ear canal and open to the ambient pressure Movement of the tympanic membrane creates a flow of air which is recorded in the flowmeter The flow is automatically time integrated, whereby the volume displacement of the tympanic membrane can be recorded direct The equipment is very sensitive and with high amplification pulse-synchronous volume changes are always recorded—even in normal ears The question arose whether these pulse volume changes are caused by variation of the caliber of the vessels in the skin lining the ear canal, in the tympanic membrane or in the middle ear mucosa and transmitted via an intact ear drum, thus making them measurable We therefore selected ears with a histologically verified glomus tumour and compared the pulse volume changes in the outer ear canal with normal ears Ears with serous otitis media were also studied because fluid is incompressible and thereby directly transmits the pulsations from the middle ear mucosa to the tympanic membrane

METHOD

Fig 1 shows a block diagram of the recording equipment It consists of a flowmeter connected airtight to the external ear canal and open to the atmospheric pressure which was kept constant The flow generated by pulsations of the vessels (V_{pulse}) is recorded in the flowmeter After amplification and integration of the flow signal it is possible to record the variation of the pulse synchronous volume from the ear canal and the middle ear (ΔV_{pulse}) directly by an ink jet recorder

The pressure chamber equipment is used to record the pulse volume changes at different positions of the drum (Fig 2) With this equipment the ambient pressure around

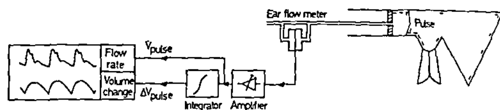


Fig 1 Equipment for recording flow rate (V_{pulse}) and volume changes (ΔV_{pulse}) in the external ear canal For details see text

the person can be varied (by up to ±15 cm H_2O) with a consequently varying degree of convexity or concavity of the tympanic membrane when the eustachian tube is kept closed The pressure of the chamber (P_{ch}) is recorded by a differential pressure transducer

With the flowmeter it is possible to record the volumetric displacement of the tympanic membrane (ΔV_{tm}) when it is exposed to different pressure gradients (Elner et al, 1971*a*)

The accuracy with which the variation of the volume is recorded is checked by means of an identical flowmeter connected to an adjustable reference volume In the situation of volume balance between the two flow systems, the flows caused by the ambient pressure variations, V_{ec} and V_{ref}, are identical (Fig 2) After subtraction in the differential amplifier the isolated recording of the flow is caused only by volumetric changes in the ear canal such as those caused by volume displacement of the tympanic membrane ΔV_{tm} The sensitivity of the flow amplifier can be adjusted in such a way that an integrated air flow volume of 0 01 μl through the flowmeter causes a deflection of 1 mm by the inkjet recorder The gas volume passing through the resistor is calibrated before and after each experiment with the aid of an airtight syringe containing 1 μl Reading accuray 1 μl ±5%

Linear response of the flowmeter system 0–300 μl/sec Dynamic sinusoidal response flat over 0–11 cps Transient response 95% 20 msec (For detailed data see Elner et al 1971*a*)

An xy recorder was used for recording the volume d

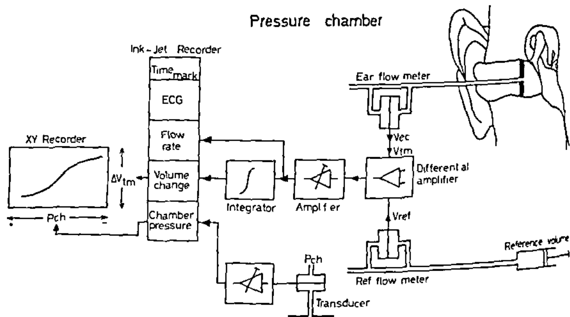
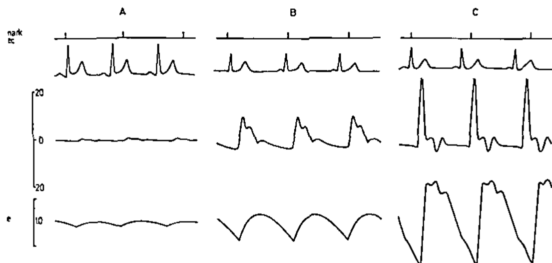


Fig 2 Equipment for recording volumetric changes in the ear canal (ΔV_{pulse}) and volume displacement of the tympanic membrane (ΔV_{tm}) in the pressure chamber
V_{tm} The air flow velocity through the ear flowmeter caused by the movement of the tympanic membrane
V_{ec} The air flow velocity through the ear flowmeter caused by compression or expansion of the gas in the ear canal and in the flowmeter system by changing the ambient pressure
V_{ref} The velocity of the flow through the reference flowmeter caused by compression or expansion of gas in the reference system by changing the ambient pressure



3 Typical recordings in a normal case (A) in serous media (B) and in glomus tumour (C) Pulse flow (V_{pulse}) pulse volume change (ΔV_{pulse}) in the ear canal and ECG are recorded simultaneously

on of the tympanic membrane with the eustachian closed as a function of the change in the pressure nber (P_{ch}) i e the volume pressure relationship of the panic membrane system (see Elner et al 1971*b*)

CLINICAL MATERIAL

material consisted of 6 patients (7 ears) with a ologically verified glomus tumour and 5 patients (5

shown in Table I

PERFORMANCE OF TEST

er microscopic inspection of the test ear a polyethylene heter (PE 190) with an individually chosen foam rubber cuff was placed in position in the bony part of the outer ear canal and checked for leakage The catheter was hermetically connected to the measuring device on which the pulse flow (V_{pulse}) pulse volume change (ΔV_{pulse}) were recorded with the drum in controlled neutral position and on some patients an ECG was also recorded simultaneously Nineteen of the 125 normal ears 2 of the 7 ears with glomus tumour and all the cases of serous otitis media were also pulse tested with the drum in different positions These positions were produced in the pressure chamber with the eustachian tube closed The test procedures were always performed with the patient sitting

ble I *Age distribution of the material*

	No of ears		
e	Normal	Glomus tumour	Serous otitis media
0	5		
-30	88		1
50	20	1	2
-60	8	2	1
	4	4	1
	125	7	5

RESULTS

Fig 3 shows typical recordings in a normal control, in serous otitis media and in glomus tumour The flow (V_{pulse}) recording resembles that of an artery pressure pulse curve and is related to the ECG recording The pulse volume change in the ear canal (ΔV_{pulse}) in the normal is 0 2 μl, in serous otitis media 0 54 μl and in the glomus case, 1 5 μl

Fig 4 shows the pulse volume changes recorded in the ear canal in normals (125) and in patients with glomus tumour (7 ears) with the drum in controlled neutral position The difference was statistically significant ($p<0$ 001) The 5 patients with serous otitis media showed pulse volume changes between 0 46 and 0 64 μl In patients with glomus tumours and ser

Table II *Symptoms and findings in the cases of glomus tumour of the middle ear*

Case	Symptoms and findings	Hearing level dB bone+air[a]	Preop ΔV_{pulse} (μl)	X ray T Tomography A Arteriography J Jugolography	Operation
I Woman age 53 Left	Pulse synchronous tinnitus 1964 Susp tumour in post inf quadrant	13/37	1 3 Neutral	T and J Neg	1967 expl tympanotomy Tumour in lower part of tympanum [illegible] adherent to drum
II Woman age 56 Left	Pulse synchronous tinnitus 1962 Susp tumour in ant and inf quadrant not in contact with ear drum	10/13	0 56 Neutral	A and J Neg	1969 expl tympanotomy Small tumour just behind [illegible] part of eustachian tube
III Man age 45 Left	Pulse synchronous tinnitus 1968 Susp tumour in post inf quadrant	10/27	0 4 Neutral 0 42 Inward 0 22 Outward	A Conglom of vessels in hypotymp and on promuntorium	1974 tumour [illegible] of tympanum Ext of bony canal [illegible] extirp of tumour [illegible] placement of the wall + myring[illegible]
IV Man age 47 Left	Pulse synchronous tinnitus 1964 1968 blue tumour except in ant sup quadrant	10/60	3 5 Neutral	J Susp glomus tumour A Tumour with vessels from a pharyng asc	1968 extirp of large tumour + radical mast[illegible]
V Woman age 55 Bilat	Pulse synchronous tinnitus bilat 1968 Both ear drums reddened	Right 38/38 Left 23/26	Right 2 4 Neutral Left 1 2 neutral	J Neg bilat A Susp tumour bilat	1969 expl tympanotomy bilat Right 2 tumours Left 1 tumour
VI[b] Man age 32 Left	Pulse synchronous tinnitus + dysphagia 1972 Red tumour in the ant + inf quadrant Paralysis of IX X XII cranial nerves left side	10/15	1 5 Neutral 1 2 Inward 0 7 Outward	T Scull base 6×5×5 destruct A blood supply from extern carotid artery	1973–75 radiotherapy and microembolization

[a] Mean value for 500 1 000 and 2 000 Hz Rel ISO (1964)
[b] Patient not in good mental condition
[c] Discharge and drum perforation
[d] Radical cavity

ous otitis media the pulse volume in the unaffected ear was $\leq 0\ 3 \mu l$ The cases of glomus tumour are listed in Table II

Fig 5 illustrates the correlation between the position of the drum and the magnitude of pulse volume in the ear canal in a normal ear In the neutral position ($P_{ch}=0$) the volume change is $0\ 1\ \mu l$ On change in the chamber pressure to +15 cm H_2O resulting in a relative underpressure in the middle ear the drum cupped inwards, and the pulse volume change was somewhat smaller ($0\ 07\ \mu l$) A corresponding change in the opposite direction (−15 cm [illegible] with a consequent relative overpressure [illegible] middle ear produced exactly the same [illegible] ($0\ 07\ \mu l$) The upper part of the curve in [illegible] denotes the mean volume displacement [illegible] tympanic membrane in 19 normal ears [illegible] different chamber pressures P_{ch} with [illegible] eustachian tube closed The lower part [illegible] figure gives the corresponding pulse v[illegible] changes in the ear canal As can be seen [illegible] is practically no difference in the mean [illegible] volume change within the range of the [illegible]

s	Postop 1976 ΔV_{pulse} (μl)
rum ıearıng us	0 ?? unchanged ın diff drum positions
ırum ıearıng rtigo	Not performed[b]
healıng rmal – One postop perf rrence	Not performed[c]
discharge ı 1974 tions rrence ged hearıng	Not performed[d]
ged hearıng ınn tus e	Right 0 75 neutral 0 45 inward 0 40 outward Left 0 90 neutral 0 90 inward 0 55 outward
ear drum ınn tus nged hearıng	0 4 unchanged ın diff positions

ent drum positions Thus, ın normal ears pulse volume change ıs the result of the lsations by the outer ear canal and not ansmitted from the middle ear mucosa vıa ear drum

Fig 7 ıllustrates the effects of drum posı ıns on the pulse volume change ın a patient ıth glomus tumour (case VI) The pulse volne change ın neutral position of the drum ıs 5 μl When the drum ıs pushed outward the ılse volume change ıs reduced to 0 7 μl and hen the drum ıs pushed ınward the corresponding volume change ıs 1 2 μl Thus, changes ın the position of the drum seem to have a marked effect on the pulse volume change ın the presence of a glomus tumour because the pulsations ın the tumour probably act dırectly upon the tympanıc membrane

In serous otitis media the pulse volume change ın the ear canal ıs also large This ıs because the fluid ın the middle ear transmits the pulsations directly from the mucosal ves sels It ıs also unaffected by variation of the pressure ın the chamber, since the drum re mains ın the same position irrespective of the pressure changes because the fluid ın the middle ear ıs incompressible

DISCUSSION

In a normal ear with an intact ear drum the pulse volume change ıs affected very little by changes ın the position of the drum This means that the pulse volume changes recorded are not transmitted from the middle ear In one control with a large pulse volume change ın the ear canal a skin lesion ın the ear canal was probably responsible for the high value (0 38 μl) This change was, however, smaller than ın the patient with a glomus tumour (case III) and ın whom the pulse volume change was the smallest (0 4 μl) among the case studied Testing at different positions of the drum ıs of considerable diagnostic value as a change ın position affects the value considerably ın the presence *of a glomus tumour, but only little ın* normals, and not at all ın patients with serous otitis media

The pulse volume change with the drum ın the neutral position varied from 0 4 to 3 5 μl ın the cases of glomus tumour The change seems to vary with the size and site of the tumour At operation, we have found a correlation between the size of the tumour and the pulse volume change The pulse volume change measured with the drum pushed outward decreased the value recorded ın all cases of glomus tumour tested ın this way Furthermore, ın these cases a slight inward move-

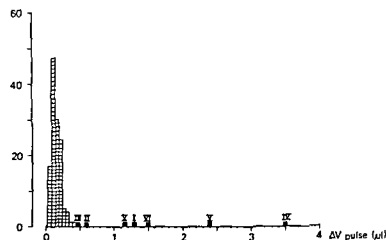


Fig 4 Pulse volume change in normal (filled columns) and in glomus tumours (unfilled columns) Case numbers according to Table II

ment from the neutral position caused a larger pulse volume change than in the neutral position In this situation the tympanic membrane comes into closer contact with the tumour and thereby increases the pulsations When the drum was moved further inward the pulse volume changes, except in case III, diminished, probably owing to an effect of changes in the elastic properties of the drum membrane

In case V postoperative testing showed abnormally large pulse volume changes The left tympanic membrane exhibited several scars, and it is probable that the pulse volume change is larger in cases with a small volume of the tympanic cavity and mastoid air cell system and with thin scars, than in a middle ear cavity and mastoid air cell system of ordinary size and with ordinary elasticity of the tympanic membrane On the right side the pulse volume change was 0 9 μl compared

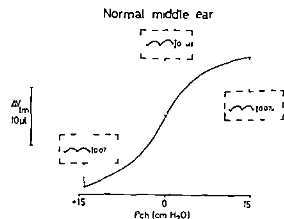
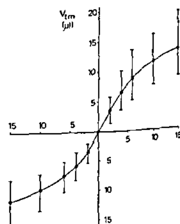


Fig 5 Effects of different drum positions on the pulse volume changes Neutral position at $P_{ch}=0$ cm H_2O (Right) the outward bulge (*left*) the inward cupping of the drum The pulse volume changes with these positions of the drum are given within the frames



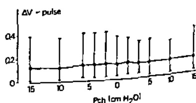
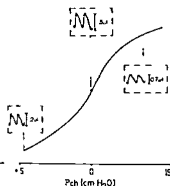


Fig 6 The upper part shows the mean compliance of the tympanic membrane system (19 ears) Neutral position in right upper corner ... volume change ... s denote ... value and the vertical bars the range



Pulse volume changes at different drum positions ase of glomus tumour (case VI) There is a large tion of the pulse volume change with the drum d outward

a preoperative value of 1 2 μl As the ent also had clinical symptoms of pulse-chronous tinnitus, there is reason to pect a recurrence of the tumour In the er part of the tympanic membrane a our is suspected but the patient is in a poor eral condition because of a coexisting gnant disease and no X ray or operation is ned

ase VI clearly shows how the method can used to follow the effect of treatment The al pulse volume was 1 5 μl and that erved after radiotherapy was 0 4 μl, which ans almost normal value

At present we are studying pulse volume nges via the outer ear canal in every case pulse synchronous tinnitus If the pulse lume change is abnormal, the pressure amber technique is used in order to confirm diagnosis Then, of course we use the thod when following up the operated cases

The technique in its present form is not itable for the general otologic clinic, but mparative studies with the impedance uipment are in progress, and we hope to pre nt a more simple method in the future In estigations on other conditions have been menced in order to find out how they fect the pulse volume change and special interest is being focused on the question what makes the pulse audible even to a normal person on physical exertion?

ACKNOWLEDGEMENT

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ZUSAMMENFASSUNG

Mit Hilfe eines Volumenstrommessers ist es möglich pulssynchrone volumetrische Veränderungen im äußeren Gehörgang festzustellen Bei 7 Ohren mit Glomustumoren in der Paukenhöhle und bei 5 mit seröser Otitis media waren diese Veränderungen größer als bei 125 Personen mit normalem Mittelohrbefund Vorausgesetzt daß der Patient nicht schluckt kann das Trommelfell durch Veränderung des Umgebungsdruckes in einer Druckkammer nach medial oder lateral verlagert werden In sämtlichen untersuchten Fällen mit Glomustumoren wurde ein beträchtlicher Einfluß von diesen Lageveränderungen auf die pulssynchronen volumetrischen Veränderungen beobachtet Bei Normalpersonen war die Veränderung gering und in den 5 Fällen mit seröser Otitis media blieb sie ganz aus Die Befunde werden dahingehend gedeutet daß die Pulsvolumenänderungen bei Normalpersonen hauptsächlich durch die Gefäße im äußeren Gehörgang erzeugt werden

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ORAL NOREPHEDRINE IN THE TREATMENT OF ACUTE OTITIS MEDI[illegible]

Results of a Double blind, Placebo controlled Trial

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(Received June 5 1977)

Abstract One hundred patients with acute otitis media were treated with oral norephedrine or with placebo for one to two weeks Symptom cards otoscopy and tympanometry were used for evaluation of the results There were no differences between the two groups with regard to acute symptoms or healing of the disease It is concluded that oral decongestants are of no value in the management of acute otitis media

Impaired tubal function is of significance for the pathophysiology and symptoms of acute otitis media, and especially for the subsequent *development of a chronic secretory otitis* media This might be the rationale for the use of intranasal or systemic decongestants in the management of otitis media Topical vasoconstrictors are difficult to apply in infants and small children In addition, they have no direct effect on the middle ear and tubal mucosa For these reasons a test of a systemic decongestant drug in the treatment of acute otitis media seems well motivated *Norephedrine* was chosen as, in contrast to ephedrine, it has little or no effect on heart and CNS when given in ordinary therapeutic doses

PATIENTS AND METHODS

The diagnosis of acute otitis media was based on the usual criteria reddened bulging oedema and immobility of the tympanic membrane In principle all patients over the age of 6 months entered the trial The following were not included (1) 10 children because it was found to be advisable to carry out paracentesis due to extreme pain or excessive fever (2) 12 children due to inadequate communication with the parents (3) patients with purulent discharge in the external ear A total of 110 patients entered the trial and the treatment was completed in 100 having acute otitis media *in 144* ears *Of these* 53 were males and 47 females The mean age was 5 3 years 6 months–40 years) The age distribution is gi[illegible] ble I

Sustained release tablets or syrup of pheny[illegible] amine hydrochloride (norephedrine) or of plac[illegible] delivered to the patients in a *randomized* ord[illegible] double blind basis The daily dosage of norephedr[illegible]

Sustained release tablet of 50 mg 1 tablet (50 mg[illegible] day for the ages 4–9 years 2 tablets (100 mg) tw[illegible] for the ages ≥10 years

Syrup 333 mg per 100 ml 5 ml (16 5 mg) th[illegible] daily for the ages 6 months – 2 years 7 5 ml (25 [illegible] times daily for the ages 3–5 years 10 ml (33 [illegible] times daily for the ages 6–9 years

[illegible]

performed and score cards collected [illegible] tion of these parameters a patient was consider[illegible] cured the treatment was *discontinued* and the pa[illegible] the trial The remaining patients were treated for[illegible] week and were examined again Treatment was st[illegible] all cases after 2 weeks but all pathological ca[illegible] [illegible] metry [illegible] ddle ea[illegible]

Table I *Age distribution of 100 patien[illegible] acute otitis media*

	No of patients	Active drug
6 months – 2 years	48	25
3 years – 5 years	27	13
6 years – 10 years	18	11
Over 10 years	7	2

·II *Mean score for ear-symptoms in pa- with acute otitis media, during and after nent with norephedrine or placebo for days (the lower the score, the milder the tom)*

	Nor ephedrine (51 pats)	Placebo (49 pats)	*t* test
a day 2+3 e 0–4)	2 16	1 90	$p>0$ 1
er of acetylsalicylic tablets used '+3	1 06	0 80	$p>0$ 1
ation of treat day 7 based ymptom scores additional therapy excellent, 2 good ubious 4 poor)	2 63	2 35	$p>0$ 1
opy day 7 re 1 5)	3 32	3 24	$p>0$ 1
anometry day 7 normal curve attened curve, egative pressure ecretion in the dle ear)	3 00	2 67	$p>0$ 1
opy day 14 re 1–5)	2 83	2 80	$p>0$ 1
anometry day 14 e supra)	2 59	2 61	$p>0$ 1

on during the period immediately before the actual tion or had been treated with tubulation of the drum use of tubal malfunction Twenty of the 100 patients given additional therapy, i e antibiotics in 13 cases entesis in 3 and antibiotics and paracentesis in 4 Of 12 were treated with norephedrine and 8 with bo

RESULTS

le II shows the symptom scores during the week of treatment and the results of scopy and tympanometry after one and af- two weeks' therapy It is obvious from this le that *norephedrine did not have even the htest beneficial effect on otitis media ptoms* As this total lack of efficacy was newhat unexpected, some of the remaining lets and syrup were tested and found to ntain active norephedrine (Lindqvist, 1977) o proper absorption of the drug was en- ed by identification of norephedrine in the ne

Possible side effects were registered in 4 cases The parents of 2 children, who got placebo, claimed that the children became sleepless during treatment In one child, 2 years old, it is possible that norephedrine aggravated a psychotic behaviour which had started one week before the trial The child was normalized the day after cessation of treatment Another 2-year-old child appeared to be dizzy during norephedrine treatment, and he got numerous suggilations of the skin These symptoms disappeared when norephedrine therapy was stopped, but this child was also treated with a fairly large dosis of acetylsalicylic acid

DISCUSSION

This trial shows that norephedrine, given in ordinary therapeutic doses, has no effect on the acute symptoms or on the healing of acute otitis media Although the objective and sensitive parameter—tympanometry—was used, there was not even the slightest trend, pointing to any difference between norephedrine and placebo Therefore, it seems highly unlikely that an increased number of patients would give a different result

A study (Aschan, 1974) has indicated that there may exist synergism between a physiological histamine antagonist (a vasoconstrictor) and a pharmacological antihistamine compound in the treatment of common cold induced nasal blockage Thus, it was a possibility that the addition of an antihistamine compound was also necessary in the treatment of acute otitis media However, Sorri et al (1977) have recently tested such a combined preparation (brompheniramine–norephedrine) in children with prolonged otitis media, and no significant differences between active and placebo treatment were disclosed de Castro (1974) gave antibiotics to 100 children and antibiotics plus a combined preparation (triprolidine hydrochloride and pseudoephedrine hydrochloride) to another 100 Based on otoscopy, 51% in the first and 70% in the second

group were considered to be cured after 10 days Although this difference is statistically significant, the result of this study is merely indicative, as the trial was not placebo controlled One large study (Rubenstein, 1967) has seriously questioned the value of oral vasoconstrictor-antihistamine agents in acute otitis media In conclusion, there seems to be no convincing evidence that peroral vasoconstrictors, either alone or in combination with antihistaminics, are of any value in the treatment of acute otitis media Topical vasoconstrictors are often used, but the practice is based on pathophysiological theory and not on controlled trials

Recently Holmquist & Larsson (1976) found that a combined preparation (promethazin and ephedrine) had a normalizing effect on tubal malfunction in adults Apparently, this is not consistent with our data or with those of Sorri et al (1977), but there may be differences in the pathophysiological basis of the diseases studied, which could account for the discrepancies

Further controlled studies are required to nfirm the significant results of Holmquist & Larsson and to further characterize the group of patients who could conceivably benefit from therapy Generally speaking, it seems necessary to perform placebo-controlled studies to reconsider the overall management of otitis media, which for many decades has been based on clinical experiences and empirical rather than rational pharmacotherapy (Diamant & Diamant, 1974)

ACKNOWLEDGEMENT

Norephedrine and placebo were kindly supplied by [illegible]
Lund Sweden

ZUSAMMENFASSUNG

Hundert Patienten mit akuter Otitis media wurden 1–2 Wochen mit Norephedrin oder Placebo per [illegible] handelt Otoskopie Tympanometrie und genau [illegible] Symptomenregister dienten zur Grundlage für die B[illegible] lung der Resultate Es konnten keinerlei Unterschi[illegible] den akuten Symptomen oder im Heilungsprozeß [illegible] den beiden Patientengruppen festgestellt werden. [illegible] ergibt sich die Folgerung daß Norephedrin per o[illegible] Wirkung in der Behandlung der akuten Otitis med[illegible]

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LABYRINTHINE AND SOMATOSENSORY CONVERGENCE UPON VESTIBULOSPINAL NEURONS

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ract In awake cats cells forming the lateral (LVST) medial (MVST) vestibulospinal tracts were identified mploying antidromic stimulation of the spinal cord ronal responses to bilateral vestibular forelimb hind and neck electrical nerve stimulation were analysed racellular recording in the vestibular nuclei was per ned via a glass micropipette saturated with Fast Green d in later histological tract identification

he number of cells projecting to cervical and lumbar ions in the dorsal and ventral division of Deiters nu s did not differ significantly An unexpectedly large nber of MVST units was found in the descending nu us Some MVST units projected to the lumbar cord but both the medial and descending nuclei projections to cervical cord were in majority Almost all spinal pro ting vestibular neurons received labyrinthine input and

licating multiple pathways As regards labyrinthine matosensory integration the two tracts were found to be ite similar The extent and complexity of labyrinthine matosensory convergence indicate the importance of d back mechanisms upon postural controls also at the vel of the vestibular nuclei

hile the vestibular labyrinth is a sensory rgan, the vestibular system must be consid red as a part of an important motor system, hich in conjunction with information derived om other than labyrinthine receptors, is vital the formation of important reflex responses he reflexes of balance The labyrinth influ nces motor adjustments via the vestibular uclei (Wilson & Yoshida 1969) which com lex (VNC) receives afferent input from the abyrinth and the somatosensory systems (Schwarz et al , 1975) as well as from several prominent descending neuronal systems involved in the regulation of spinal motor activity (Nyberg Hansen, 1966)

There are two major fiber systems projecting from the VNC to the spinal cord the lateral vestibulospinal tract (LVST), arising in the lateral vestibular nucleus (LVN) and the medial vestibulospinal tract (MVST) originating in essentially the medial (MVN) but also in adjacent parts of the descending (DVN) and lateral (LVN) vestibular nuclei (Nyberg Hansen, 1966, Wilson 1972, Akaike, 1973, Peterson & Coulter, 1977)

Pompeiano & Brodal (1957) demonstrated that the LVST was somatotopically organized and a similar organization has been shown for the MVST by Wilson & Yoshida (1969) In the pigeon, Rabin (1975) pointed out that the association of the labyrinth with limb muscles is insignificant when compared with the association with neck muscles The importance of neck–vestibular integration in the cat VNC has been emphasized by Rubin et al (1975)

However other investigators have indicated that in the cat (Diete Spiff et al , 1967) and the rabbit (Akaike et al , 1973) stimulation of the labyrinth has a profound effect on motor activity at all levels of the cord Would one thus expect somatosensory input to the spinal projecting VNC units from all parts of the body equally or would some parts be less repre

This project was supported by the Canadian Medical Research Council (grant MA 3311) and by the Swedish Medical Research Council (grant 14X-4503)

sented? Somatosensory input to the VNC has been shown to be quite extensive (Allen et al , 1972*a*, 1972*b*, Schwarz et al , 1975, Ten Bruggencate et al , 1975) but little information concerning specific labyrinthine and somatosensory input to cord projecting units in cat is available In the present study, electrophysiological techniques have been employed to investigate the influence of somatosensory and labyrinthine inputs on VNC neurons projecting to the spinal cord

MATERIALS AND METHODS

The experimental series included 19 adult cats Surgery was performed under halothane–N_2O–O_2 anaesthesia Tracheotomy and artificial respiration were routinely employed End-tidal pCO_2 was monitored continuously with a Beckman Gas Analyzer and kept at 3 5 to 4 5% A constant body temperature, 37–38°C was maintained throughout each experiment The halothane administration was stopped prior to recording and the animal immobilized with gallamine triethiodide (Flaxedil) Wounds , pressure points had previously been in- , rated with Xylocaine, and this local anaesthetic was given repeatedly during the recording Absence of piloerection and presence of complete miosis indicated absence of pain

Bipolar silver wires (125 μm insulated diameter) were positioned on the anterior branches of the ipsilateral (with respect to the recording side) and contralateral vestibular nerves (VN), via an inferior approach to the labyrinth Identical electrodes were placed on the cochlear nerve within the modiolus and on the facial nerve at its lateral genu Current spread was controlled by threshold comparison for these 3 electrode pairs, observing evoked eye deviation and neural activity in the VNC Electrode isolation was secured by paraffin

The sciatic nerve, the radial nerve and the nerves from C2–C3 to the cleidotrapezoid muscle were dissected bilaterally and mounted on plastic 'rails' containing bipolar stimulating steel electrodes No peripheral nerve w Manual stimulation (touch rotation of pressure to muscles etc) of differen parts gave additional information conc types of somatosensory input to the an cells

The C2–C4 segments as well as the l segments were exposed through a dor minectomy An insulated silver wire (1 exposed at the tip was placed on the surface of the cervical cord near the mid the C3–C4 level A second wire was h under the cord and placed on the ventr face A similar procedure was perform the L3–L4 level, thus permitting bipolar lation of the different spinal segments

Following posterior craniotomy the a was placed in a David Kopf stere frame A recording glass micropipette NaCl, 1–3MΩ) saturated with Fast (FCF was introduced obliquely into the stem above the obex At the bottom of track dye was iontophoretically ejecte mark the location of tip (Thomas & Wi 1965) The VNC was reached without p trating the cerebellum The placement o electrode in the VNC was based upon ste taxic coordinates and the physiological nique of Shimazu & Precht (1965)

The electrical nerve stimulus consiste square waves (0 1 msec duration) with an tensity 1 5 times threshold (evoked eye de tion and muscle twitches respectively) frequency was usually 1 stim/sec

Unitary spike activity and field potent were displayed on the oscilloscope after c ventional a c amplification (band width Hz – 300 Hz for field potentials 100 Hz t kHz for spike potentials) Additional analy of unitary activity was made with a laborat computer (PDP 8E, peristimulus time his grams, adjustable bin widths for 100 bins i histogram)

Units in the VNC projecting to the ce were identified by antidromic stimulation cervical and lumbar levels It was of prime i portance that only certain criteria were a

Table I *Projection of vestibulospinal units*

Nucleus	To cervical cord (C cells)	To lumbar cord (L cells)
LVN	31	32
VLVN	19	15
DLVN	12	17
MVN	41	7
DVN	15	1
Total	87	40

tested for antidromic activation (Kelly et al, 1975, Fuller & Schlag, 1976)

Once a unit was identified as a projecting unit its spontaneous activity (if any) was recorded The neuron was then subjected to a battery of stimuli, the response to bilateral vestibular, neck, radial and sciatic nerve stimulation was investigated as was the response to manual somatosensory manipulation

At the end of the recording session the cat was killed with a lethal dose of Nembutal and perfused with 10% formaldehyde The brain stem was removed, frozen, and serial transverse sections were cut at 100 μm The dye marks could be identified in these sections which were stained by the Kluver Barrera method The various nuclei were delineated according to Berman (1968) Cells which were found to lie on or near nuclear boundaries (especially within LVN) were rejected in order to minimize the inclusion of any LVST projecting units from other than LVN and of MVST units from other than MVN or DVN

RESULTS

A total of 127 neurons projecting to the spinal cord were included Based upon the results of various investigators (see Pompeiano 1972 for a review) units located clearly within the borders of the LVN sending axons to the spinal cord were considered part of the LVST and neurons lying clearly within the boundaries of the MVN and DVN were correspondingly included into the MVST As shown in Table I the largest part of projecting units was found in the LVN The antidromic spikes recorded in the LVN had latencies ranging from 0 8 to 1 7 msec for cervical stimulation and 2 4 to 5 3 msec for lumbar stimulation

While cervical cord projecting units (C cells) predominated overall, lumbar cord projecting units (L cells) were most frequent in the DLVN There was a small difference in the numbers of C and L cells in VLVN and DLVN (ventral and dorsal parts of the LVN)

MVST units fired their antidromic spikes with latency range of 0 5–3 msec In both the MVN and DVN the projection to the cervical cord far outnumbered the projection to the lumbar cord—an indication of the importance of this tract in orientation of the upper body As noted in Table I an interesting finding is the relatively large number of spinal projecting neurons in the DVN There also appeared to exist direct pathways, although few, between MVST units and the lumbar cord

The superior vestibular nucleus (SVN) completely lacked neurons projecting to the cord judged from a sample of 203 units

Labyrinthine Input to Spinal Projecting Units

As indicated in Table II some units projecting to the spinal cord received no labyrinthine input However, the vast majority (85%, $n=127$) received input from labyrinthine receptors The units in the MVN and in the DVN, showed *predominantly bilateral labyrinthine* influence (54%, $n=48$ for MVN, 56%, $n=16$ for DVN) The projecting LVN units, however, manifested a preference for ipsilateral labyrinthine input (68%, $n=63$) No vestibulospinal unit with only contralateral labyrinthine input was ever encountered The MVST units of the DVN were found to contain the largest percentage (25% $n=16$) of units not responding to labyrinthine stimulation, the small sample, however, makes conclusive statements impossible

Neurons influenced by ipsilateral labyrinthine stimulus were analysed as regards their

Table 2 *Labyrinthine and somatosensory input to vestibulospinal VNC units*

Abbreviations DVN=descending vestibular nucleus LVN=lateral vestibular nucleus LVST lateral vestibulosp tract MVN=medial vestibular nucleus MVST=medial vestibulospinal tract SI=somatosensory input VNC… bular nuclear complex

	MVN Units		DVN Units		LVN Units		
	SI	no SI	SI	no SI	SI	no SI	Total
From ipsilateral labyrinth only	6	8	2	1	19	24	60
From contralateral labyrinth only	0	0	0	0	0	0	0
From both labyrinths	23	3	7	2	12	2	49
No labyrinthine input	8	0	4	0	6	0	18
Total	37	11	13	3	37	26	127

response latencies Due to the difficulty of determining exactly where an inhibition begins, bin widths of 0 5 msec were employed (Fig 1) for inhibited units These units often also showed an excitatory response which is included in the excitatory response data In order to separate monosynaptic from disynaptic and polysynaptic excitatory responses, latencies were examined using bin widths of 0 1 msec (Fig 1 A) Employing the monosynaptic latency grouping of Wilson et al (1967), all units discharging within the latency range of 8–1 5 msec were classified as monosynaptic (all spikes that occurred 0–0 6 msec after the start of the N_1 potential) Units with a response latency of 1 6 to 2 2 msec were classified as disynaptic (Ito et al , 1969) Those with latencies 2 2 msec were considered polysynaptic

Monosynaptic units were found for both LVST and MVST systems The LVST units appeared to fall into three major latency populations (Fig 1 A) One population showed monosynaptic responses centered around 1 1 msec, a second around 2 9 msec while a third one was concentrated around 4 msec The MVST units appeared to contain only two latency populations The early monosynaptic one coincided with that of the LVST units and the second population centered around 2 5 msec No spinal projecting units were encountered answering to ipsilateral vestibular stimulus with a disynaptic latency

An interesting finding was the relatively large number of units inhibited by laby input (Fig 1B) The earliest inhibitory response was 3 msec for both LVST and M units As was seen for excitatory resp the inhibitory ones fell into distinct lat groups LVST neurons centered around

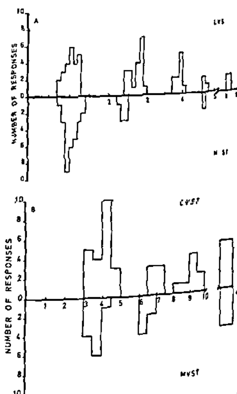


Fig 1 Latency histograms for ipsilateral labyrinth to vestibulospinal cells (A) Excitatory LVST res are indicated above the MVST below the x-ax widths of 0 1 msec were employed up to 5 msec hibitory responses are similarly displayed B in 0 5 msec were employed up to 10 msec

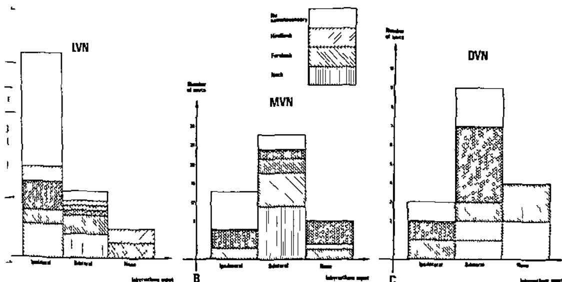


Fig 2 The distribution of labyrinth and somatosensory input to the vestibulospinal cells The units were divided into three groups based on labyrinth input ipsilateral bilateral and no labyrinth input Units receiving solely contralateral labyrinth input were absent For each column the somatosensory input patterns and proportions are indicated (A) Units in the lateral vestibular nucleus (LVN) (B) Units in the medial vestibular nucleus (MVN) (C) Units in the descending vestibular nucleus (DVN)

nd 9 msec while in the MVST population latencies gathered around 4 and 6 5 msec

Somatosensory Input to Vestibulospinal Units

(a) LVST

Somatosensory input was noted in 58% (n=63) of the LVST units and 49% displayed somatosensory and labyrinthine convergence To facilitate analysis of this convergence, LVST units were divided into four categories based upon the pattern of labyrinth input demonstrated in Table II Most LVST units received an ipsilateral labyrinth input Of these, 56% (n=43) received no somatosensory input while the remainders had somatosensory receptive fields most frequently engaging forelimb regions (27%, n=19) or larger body areas (neck forelimb and hindlimb, 32% n=19) (Fig 2A) Twenty two per cent of the LVST cells received bilateral labyrinth input and 86% (n=14) of these responded to peripheral nerve stimulus and/or manual somatosensory activation

Of the 63 LVST units 6 did not respond to labyrinth input but could all be demonstrated to receive somatosensory input Thus all LVST units received labyrinth and/or somatosensory input The LVN neuron in Fig 3 belongs to the group influenced by both labyrinths but with no proven somatosensory input

(b) MVST

MVN 77% (n=48) of the units responded to neck or limb nerve stimulation and in 60% a labyrinth–somatosensory convergence was demonstrated Of all MVN units, 23% responded to labyrinth input exclusively and 17% were affected only by somatosensory input No MVN units were devoid of afferent input (Table II) Activation from both labyrinths occurred for 53% of MVN cells and of these neurons somatosensory activation was noted in 88% (n=26) The most common somatosensory response (48%, n=23) was seen following neck nerve stimulation (Fig 2B) Fig 3 (D–F) illustrates a convergence

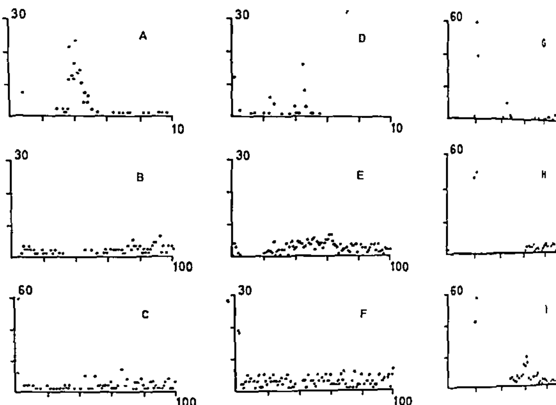


Fig 3 Response latencies of three representative spinal projecting vestibular neurons (A–C) LVST unit responding only to vestibular stimulation with ipsilateral excitation (A) and contralateral inhibition (B) (C) shows resting discharge (D–F) MVST unit in the medial vestibular nucleus showing labyrinthine/neck convergence with an excitatory response to ipsilateral labyrinth (D) and inhibition to ipsilateral neck stimulation (E) (F) is spont activity (G–I) MVST unit in the descending ves nucleus receiving convergent input from labyrin neck (G) is the excitatory response to ipsilatera tibular stimulation (H) the inhibitory influence of c lateral neck stimulation while an ipsilateral one p same type of response but with a shorter la

MVN neuron being excited by ipsilateral labyrinth stimulation and inhibited by ipsilateral (C_2–C_3) neck nerve stimulation

As noted above for LVST units, no MVST units were recorded which responded solely to contralateral vestibular stimulus An ipsilateral input was seen in 29%, but in contrast with data in the "bilateral" group, the majority (57%, $n=14$) received no somatosensory input (Table II, Fig 2B) When labyrinthine-somatosensory convergence was observed the somatosensory receptive fields usually included neck and limb areas in both body halves (Fig 2B) Unlike the LVST, no unit in MVN or DVN responded to only sciatic nerve stimulation

DVN 81% ($n=16$) had somatosensory input, 56% showed labyrinth–somatose convergence Twenty-five per cent of the were influenced by somatosensory input No MVST units were free of afferent (Table II; Fig 2C) The largest group w tivated from both labyrinths (56%, $n=$ this group 7 units were convergent unit also received somatosensory input The common somatosensory response fol neck, forelimb and hindlimb stimuli (Fig No units had contralateral labyrinth only. Fig 3 (G–I) shows a DVN unit convergence of ipsilateral labyrinth inp sponding monosynaptically) with bil neck input Due to small sample of units ments regarding representative types of tosensory input patterns are impossible

DISCUSSION

ny anatomical and physiological studies e confirmed the existence of two ma- vestibulospinal projection systems The ST is acknowledged to arise almost exclu- ly from Deiters' nucleus (LVN) while the 'ST has been shown to have its origin in the 'N and adjacent DVN and perhaps LVN yberg Hansen 1966, Wilson, 1972, Akaike, 3 Peterson & Coulter, 1977) By restricting analysis to those units which lie clearly hin the boundaries of each nucleus, very v MVN or DVN units included here are ely to project via the LVST Similarly, the elihood of LVN units examined projecting o the MVST is minimized

The present study has shown that vestibular its often receive different patterns of laby ith–somatosensory input depending upon e nucleus of origin

The basic assumption has previously been at the contribution of VLVN and DLVN its to the LVST did not differ significantly Vilson 1972) The present data based on a omparable number of electrode tracks and nalysed neurons in the two nuclear subparti ons support this observation (Table I) The rojection to spinal levels is however differ nt a finding also noted by Wilson et al 1967)

It would appear that there is a larger projec on of the DLVN to the hindlimb control reas of the cord The VLVN appears to show greater projection to upper body areas— eck and forelimbs—but also contains many indlimb projecting units The projections to he cervical and lumbar cord are separate However, Abzug et al (1973), have found many LVN units influencing both lower cervi cal and lumbosacral regions of the spinal cord If branches of LVST axons act on both fore- limb and hindlimb motoneurons they will im pose a degree of forelimb–hindlimb coordina- tion in addition to the forelimb–hindlimb re flexes that are present In this study LVST branching units were not studied

Inhibitory responses to labyrinth input were commonly noted for LVN cells Such responses were absent in the study by Wilson & Felpel (1972) It is possible that the presence of an intact cerebellum was responsible for the inhibitory responses (Ryu & McCabe, 1976)

Slightly more than half of the LVST units (37/63) had a somatosensory input and almost one half (31/63) showed labyrinth/somatosen sory convergence The LVST regulates motor activity through di and polysynaptic connec tions (Hongo et al , 1975) with alpha and gamma motoneurons and by modulating seg mental reflexes by means of terminations on interneurons in their pathway (see Grillner, 1972, for a review) Such a system is highly dependent on feedback information which is il lustrated by the large percentage of somato- sensory afferents seen in this study

Certain LVST units can also be activated by labyrinthine impulses Furthermore, they are under the control of cerebellum and of im pulses of somatic and other origins (Wilson, 1972) making coordination of this complex system possible LVST units are subsequently not simple relay units for labyrinthine com mands to the spinal cord motoneurons but they receive somatosensory information for feedback (to spinal cord) and feedforward (to higher centers of CNS) processes, as well as labyrinth–somatosensory convergence in or- der to integrate information from different mo- dalities

The majority of MVST units in the present study were antidromically activated by cervi- cal cord stimulation Stimulation of the MVN and DVN may influence the lumbar segments of the spinal cord bilaterally (Pompeiano, 1972) As a result, two main pathways have been described through which vestibular im pulses may reach the lumbar cord (1) via vestibuloreticular fibers from the MVN and DVN (Ladpli & Brodal, 1968) relayed to the cord via bilateral reticulospinal fibers which can reach the lower spinal segments (Nyberg Hansen 1966), (2) via descending propriospi-

nal fibers interconnecting the spinal enlargements (Giovanelli & Kuypers 1969)

However, the above two pathways act on the lower cord via synaptic pathways whereas in the present study some of the MVST units appeared to project directly to the lumbar cord Most of these MVST units displayed extremely rapid labyrinthine responses (3 msec) In fact, more than half of the neurons activated by ipsilateral labyrinth stimulation showed monosynaptic discharge Wilson et al (1968) also found when employing decerebellate cats that among cells projecting to the MVST, a greater percentage received monosynaptic input than any other group of cells studied in the MVN

The MVST system is considered by many authors to reach no further caudally than to the mid thoracic cord (Brodal et al , 1962, see however, Akaike, 1973) and to have a strong direct input from labyrinthine receptors (Wilson et al , 1967) Except for the minor contingent of MVST units projecting to the upper cord the present findings are in agree with these opinions These findings together with the data that the cervico thoracic component of the LVST is relatively more subject to labyrinthine control than the lumbosacral component (see Wilson 1972 for a review) explain the observations that the motor activity of the cervicothoracic cord is more influenced by the labyrinth than motor activities of the lumbosacral cord However, it must be emphasized that the MVST action on the cervicothoracic cord is particularly directed towards neck motoneurons rather than forelimb motoneurons

Most of the MVST units showed labyrinth–somatosensory convergence with somatosensory receptive fields frequency in upper parts of the body Yet, many of these convergent units also responded to hindlimb input Mori & Matsumoto (1972) have demonstrated the existence of a system within the spinal cord transmitting input from the neck to forelimb motoneurons and modulating the excitability of the flexor reflex It is quite likely that such a system also exists for neck–hindl[illegible] forelimb–hindlimb coordination [illegible] limb adjustments are part of the overall [illegible]tural changes when the balance of the [illegible] is upset (Roberts 1967)

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It can be concluded that the LVST [illegible] MVST units show a difference with regard not only their site of origin but also [illegible]ing the response characteristics to multimod[illegible] stimuli

ZUSAMMENFASSUNG

Bei wachen Katzen wurden die Neuronen die de[illegible]teralen (LVST) und medialen (MVST) Tractus vesti[illegible] spinalis bilden durch antidrome Stimulation des Rü[illegible]

wurden analysiert Eine extrazellulare Registrierung [illegible] Glasmikropipetten gefullt mit Fast green wurde [illegible] Vestibularkernen durchgefuhrt um eine spatere [illegible]logische Identifikation moglich zu machen Es gab [illegible] signifikativen Unterschied zwischen dem dorsalen [illegible] ventralen Teil von Deiters Kern in bezug auf die [illegible] Neuronen welche Reizantworten in der Hals- und L[illegible]denregion auslosten Eine unerwartet große Anzahl [illegible] MVST Neuronen wurde im kaudalen Kern gef[illegible] Einige MVST Neuronen losten im Lendrückenmark R[illegible]antworten aus aber sowohl der mediale als auch der [illegible]dale Kern lösten uberwiegend Reizantworten in der H[illegible]region des Ruckenmarks aus Beinahe alle Vestibu[illegible]ronen die im Ruckenmark Reizantworten auslösen [illegible]hielten Innerohreinfluß und mehr als die Halfte [illegible]sensorischen Einfluß Die Neuronen konnten auf Gr[illegible] unterschiedlicher erregender und hemmender Antwor[illegible]tenzen des Labyrinths in verschiedene Gruppen auf[illegible] werden die auf mehrfache Wege schließen lassen [illegible] die Integration der Labyrinthbahnen und der [illegible]sensorischen Bahnen betrifft erweisen sich beide als [illegible]standig gleichwertig Der Umfang und die Komple[illegible] der Labyrinth–somatosensorischen Konvergenz er[illegible] die Bedeutung von Ruckkopplungsmechanismen fü[illegible] Raumlagekontrolle auch auf dem Niveau der Vestib[illegible]kerne

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ZUSAMMENFASSUNG

Bei wachen Katzen wurden die Neuronen die

trische Reizung des Vestibularnervs auf beiden der Nerven der Vorder und Hinterbeine sowie des wurden analysiert Eine extrazellulare Registrier Glasmikropipetten gefüllt mit "Fast green" wurde Vestibularkernen durchgeführt um eine spätere logische Identifikation möglich zu machen Es gab signifikativen Unterschied zwischen dem dorsal ventralen Teil von Deiters Kern in bezug auf die Neuronen welche Reizantworten in der Hals- und denregion auslösten Eine unerwartet große Anza MVST Neuronen wurde im kaudalen Kern ge Einige MVST Neuronen lösten im Lendruckenmar antworten aus aber sowohl der mediale als auch d dale Kern losten uberwiegend Reizantworten in der region des Ruckenmarks aus Beinahe alle Vestibul ... auslös ... älfte ... n auf ... r Ant ... pen au

tenzen des Labyrinths in verschiedene ... werden die auf mehrfache Wege schließen lassen die Integration der Labyrinthbahnen und der s sensorischen Bahnen betrifft erwiesen sich beide standig gleichwertig Der Umfang und die Konv der Labyrinth–somatosensorischen Konvergenz die Bedeutung von Ruckkopplungsmechanismen Raumlagekontrolle auch auf dem Niveau der Vest kerne

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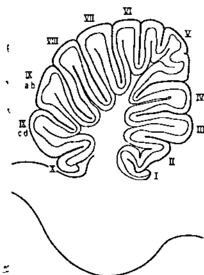


Fig. 1. Diagram of a sagittal section of the cerebellar vermis of a pigeon. Lobules are numbered according to Larsell (1967). The stippled area indicates the region explored with microelectrodes.

ated by a constant gas stream through a tracheostoma and a cannulation of the abdominal air sack and paralysed with Flaxedil.

The head was clamped in a stereotactic frame (a modification of Karten & Hodos 1967) device to permit a more rigid fixation in pitch) which was mounted on a rotation table with the center of the interaural axis coincident with the center of rotation. Position of lateral semicircular canals was adjusted to be approximately coplanar with the rotation plane, it could, however, be tilted laterally (in roll) out of this plane by up to 40° to either side. The body was placed into a snugly fitting plaster of Paris cast mounted on a metal bar which could be rotated about three axes (roll, pitch and yaw) with respect to the head, the corresponding joints being located just under the pigeon's head. The yaw axis joint was attached to a potentiometer so that neck joint position could be monitored. During rotation of the table, body position was always fixed with respect to the head, as was the tilt angle of the head with respect to the table. The axis of the table was directly mounted to a servo-controlled torque motor driven by a function generator to provide sinusoidal rotations of 0.25 or 0.5 Hz with maximal angular velocities of ca 60°/sec.

The bone overlying cerebellar lobules VI, VII and VIII (Larsell, 1967) was removed and the dura mater incised to permit systematic scanning of the underlying cerebellar cortex (Fig. 1) for neurons responding to rotation in the horizontal canal plane. The halothane anaesthesia was discontinued prior to recording, leaving the animal under the analgesic action of N_2O and Ketamine. Extracellular neuronal activity was recorded with glass micropipettes filled with 2 M NaCl (impedance 1–5 MΩ at 1 kHz). These electrodes were driven vertically (with respect to horizontal canal plane) through the cortex, the cortical layers being roughly identified by background activity patterns. During penetration, the preparation was subjected to manual rotation in the horizontal plane until responsive neurons were isolated. Each of these neurons was further investigated with controlled horizontal rotatory stimuli with the head held horizontally or at a lateral angle of up to 30°. Responses to lateral tilting movements (about the

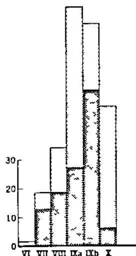


Fig. 2. This histogram indicates the relative numbers of cells in lobules VI through X which responded to vestibular (light stippling) or both vestibular and neck (dark stippling) stimuli. Lobule "IXa" includes IXa and b, "IXb" includes IXc and d according to Larsell (1967).

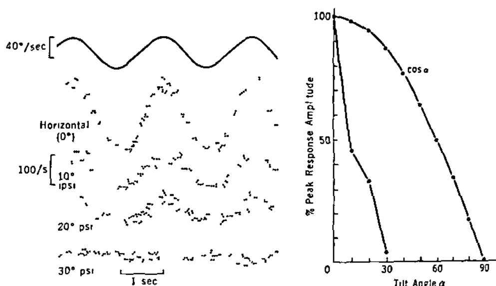


Fig 3 Histograms represent the responses of a neuron to 16 identical horizontal rotations at four different tilt angles (0°, 10°, 20°, and 30° tilt with ipsilateral side down) Head velocity is shown in the top trace The diagram on the right is a plot of the response amplitude of this cell as function of tilt angle The theoretical cosine response also plotted for comparison

roll axis) were also noted Then the body was manually moved about each of the three available neck rotation axes in search for neuronal esponses

)Conventional a c recording techniques with a bandwidth of 1 Hz to 10 kHz were used Spikes of identified neurons were filtered at 300 Hz and transformed, by means of a window discriminator, into standard pulses These were counted by computer to produce stimulus time histograms for a number of identical rotatory sinusoidal stimulation cycles (usually 16) Standard pulses were also used to compose instantaneous diagrams of frequency versus time for manual stimuli which were not suitable for averaging techniques

During each experiment a number of electrode tips were left *in situ* so that tip positions in histological slides could be compared with the same positions determined electrophysiologically For this purpose the brains were perfused transcardially under halothane anaesthesia with a fixative the cerebella cut at 90 μm on a freezing microtome and stained with cresyl violet Since there was a good agreement between these techniques, the histological location for each investigated neuron could be extrapolated from electrophysiological data for all other recording locations

RESULTS

Since it had been shown in a previous study that neck and labyrinthine impulses converge in lobules VII, VIII, IX and X of the cat cerebellum (Schwarz et al , in press) neurons were sampled throughout the region illustrated in Fig 1 This distribution of convergence was confirmed in the present study, employing natural stimuli for both afferent systems Fig 2 illustrates the distribution of vestibular (light

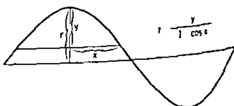
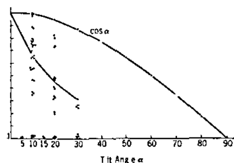


Fig 4 This diagram explains the equation used to calculate the response amplitude when only a portion of the sinewave was present r = Theoretical response amplitude x = 1/2 response duration expressed as an angle y = observed response amplitude



5 Diagram of the response amplitude at different angles expressed as a percentage of the peak response population of cerebellar cells The predicted cosine relationship is plotted for comparison The solid line connects the mean response amplitudes at tilt angles of 10°, 20° and 30°

pling) and vestibulo cervical (dark stip g neuronal responses in these lobules This ogram includes all vestibular responses, only those classified as horizontal canal sponses and described in detail below, however, neck input is only included when it con ges with vestibular input It is remarkable t very few neck afferents appear to be ected to the nodulus (5%), whereas most re seen in the uvula (68%) This matches e pattern seen in the cat (Schwarz & Milne, 1976) Direct vestibular fibers are directed y to the ventral portion of the uvula and the nodulus (Whitlock, 1952, Schwarz & hwarz, in preparation)

The histogram of Fig 2 is biased towards semicircular canal responses since ly cells responding to horizontal rotation re searched for detailed study As we shed to select units specifically responding horizontal canal stimulation, criteria for this put had to be adopted We believed initially at the following three criteria would select e desired neurons

1) the cell should not respond to lateral tilt- g movements

2) the phase angle between stimulus in the horizontal plane and response should indicate reasonable correlation between unitary activity and head velocity (cf Goldberg & Fernandez, 1971, Lifschitz, 1973, Blanks et al, 1975),

3) when the pigeon's head was tilted about the roll axis the amplitude of the neuron's response to horizontal rotation should diminish according to a cosine function (cf Blanks et al, 1975)

While there were many neurons to satisfy the first two criteria, not one was found acceptable according to the third one

An example is given in Fig 3 This neuron exhibited no detectable change in background activity with angular acceleration about the roll axis The velocity of horizontal rotation is shown on top The four histograms below show responses to horizontal rotation when the head was positioned horizontally and tilted by 10°, 20° and 30° towards the ipsilateral side A similar response series was seen with contralateral tilt It is evident that modulation of background activity is approximately in phase with head velocity and that response ampli-

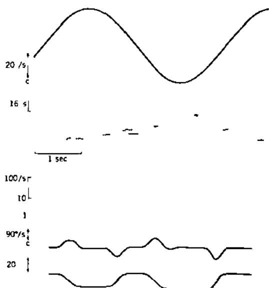


Fig 6 Responses of a neuron to head and neck rotation Top trace=head velocity second trace=histogram of response to sixteen identical head rotations third trace=instantaneous firing frequency in response to rotation of the neck fourth and fifth traces represent neck velocity and position respectively i=Ipsilateral c=contralateral

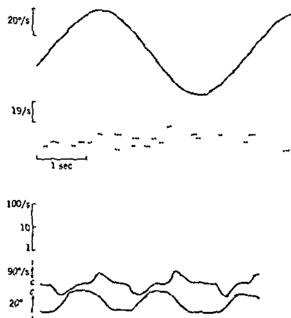


Fig 7 Responses of a neuron to head and neck rotation Top trace=head velocity, second trace=histogram of response to sixteen identical head rotations third trace= instantaneous firing frequency in response to rotation of the neck fourth and fifth traces represent neck velocity and position respectively

decline with tilt about the roll axis However, the amount of response decline is far greater than predicted by the cosine function For example, 87% of the greatest response amplitude should still be present with a 30° tilt according to that function, whereas no response is present in the bottom histogram In the diagram to the right the response decline seen is compared with that predicted by the cosine relationship Evidently this cell is much more specifically tuned to the horizontal plane than a primary horizontal canal cell should be

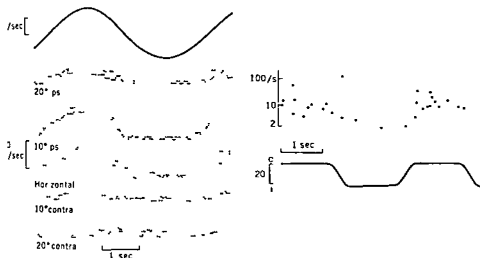
The cell of Fig 3 was not typical for our population of 96 neurons receiving horizontal canal input (so classified in spite of criterion 3) since its response represented an almost complete sinusoid For most cells only a segment of the sinewave was present in the response (e g Fig 8) As Fig 4 shows, the amplitude and duration of such a segment can be used to examine the validity of the cosine relationship for cerebellar neurons responding to semicircular canal input The peak of the sine

wave obtainable by regression from a h[...] gram containing only a sinewave segm[...] be obtained using the relationship to th[...] of Fig 4, where r is peak amplitude, y o[...] served amplitude and x half of the re[...] duration expressed as an angle

Peak amplitudes of response sinusoid[...] tained for many units according to Fig[...] different tilt angles are summarized in [...] where they are expressed as percent[...] the maximal amplitude obtained in the [...] zontal plane It is clear that all amplitu[...] angles between 5° and 30° are smaller[...] they should be according to the cosine[...] tionship indicated in this graph A sol[...] connects the means at 10°, 20° and [...] lustrating a considerably greater plane[...] cificity for the cerebellar neuron popu[...] than for known primary afferents

Since the horizontal canal plane is mo[...] cisely represented in the cerebellum tha[...] tulated by our third criterion we accep[...] "horizontal canal cells" all those cells [...] fying the first two criteria and exhibiting[...] maximal response amplitude in the hori[...] plane The vast majority of these ce[...] were recorded from the granular laye[...] only a few from the neighbourhood of th[...] kinje cell (PC) layer (in our laboratory P[...] not be identified without antidromic [...] stimulation since interneurons are al[...] pected to produce complex spikes) T[...] sponsive cells were found in the [...] layer where extracellular spikes had long[...] tions (5–40 msec) (cf Llinas & Nich[...] 1969 for an interpretation) and fired wi[...] background frequency (below 2 Hz) [...] horizontal canal cells, 41 responded to [...] rotation as well All of these neuro[...] sponded to neck movement about the ya[...] with only 3 giving clear although weake[...] sponses to roll movements as well [...] changed their firing rate with pitch [...] ments

Examples are given in Figs 6 to 8 [...] zontal canal cell of Fig 6 exhibits a [...] tonic response to ipsilateral movements [...]



8 Histograms on the left show responses to horizon[tal] rotation at five different tilt angles (upper trace= velocity) Diagram on the right illustrates the response of this cell to neck rotation (lower trace=neck position

y with respect to the head, thus signalling k position The vestibular response en- es head velocity towards the contralateral Since contralateral body movements can nce the cell modulation of activity due to ibular stimulation acts upon a background en by neck position Few such purely tonic ponses were seen The neuron of Fig 7 ponds in a purely phasic fashion to ipsi- ral neck movements in yaw, signalling a nge in neck position This phasic neck re- nse can easily override the vestibular signal ut head velocity towards the contralateral e when the pigeon moves its head under ural conditions This type of neuron may be ated to vestibular nuclei cells, which have n shown to code both neck and head velo- y (Rubin et al , 1975) Unfortunately the nual analysis techniques available for neck ponses in this study did not permit a deci- n as to how well the firing pattern of phasic ls corresponded to velocity

Responses of all 41 neurons to yaw neck vements were directionally specific, neck nding towards ipsilateral being excitatory d towards contralateral inhibitory or vice rsa The directional specificity encoded in ck responses did not, however, match that displayed in the horizontal canal responses in a systematic fashion about as many activations in yaw towards ipsilateral converged with canal activation due to ipsilateral head rotation as with activations due to rotations towards the contralateral side That is to say, the chances that information from the neck is cancelled by the labyrinth due to subtraction when the pigeon moves its head towards one side are about as great as those of the generation of a stronger signal due to summation of both inputs

Fig 8 shows an aberration from the normal response pattern seen only rarely in this study (4 cells) The maximal amplitude to rotation is seen when the head is tilted by 10° ipsilaterally rather than in the horizontal plane All other neurons recorded during this experiment exhibited the usual maximum in the horizontal plane It will be recognized that in all other aspects this neuron reacted like horizontal canal cells, encoding head velocity and not responding to tilt movements in roll The neck response is an activation during a contralateral yaw deviation

It is noteworthy that the neuron population described thus far represents a carefully biased sample Many neurons responding to tilt about

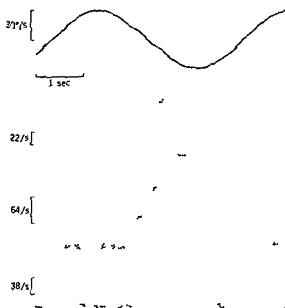


Fig 9 Responses of four d fferent cells to honzontal sinuso dal rotation Histograms represent responses to *sixteen ident cal* st muli head velocity is illustrated in the top trace

the roll axis *were not analysed* in detail It is *interesting to note* however that all these *neurons* responded to pitching or rolling neck ovements with very few changing their ac vity during yaw movements provided neck input was present at all

Many neuronal responses to horizontal head rotation did not justify classification into the *horizontal* canal group although the hori *zontal canals* may well be involved in their generation Examples for four different cells are given in Fig 9 The first histogram has two peaks of excitation one in phase with head velocity and one leading (or lagging) head velocity by ca 90° A convergence of a velocity and position coding element could ac count for this pattern (e g convergence of a *primary* canal afferent with an indirect canal *afferent* after integration) The response il lustrated in the second histogram might (or might not) be interpreted as coding position which could of course also be caused by otolithic input A curious trapezoidally shaped pattern such as in the third histogram was seen quite often This response is not a clipped segment of a sinusoid since smaller re sponses caused by lateral tilt had th trapezoidal shape Finally it should forgotten that in almost all stud es of th ture a number of entirely uninterpreta sponse patterns can be seen such as th the last histogram

DISCUSSION

Two lines of evidence emerging from th reported above as well as from earlie (Schwarz & Tomlinson 1977) indicate t *cerebellar* cortex receives rather spe formation about movement direction signalling horizontal head rotation is mo cise than available from known first or teral semicircular canal afferents and th ferents specifically converge with som sory afferents informing about neck ments in a corresponding plane

No mechanism is known that can greater precision for vestibular activ nalling the plane of horizontal head than given by a cosine function Th s f describes the amount of canal stimula any given plane of rotation and has been shown to be an accurate descripti few semicircular canal afferents in *(Blanks et al* 1975) According to th tion neuronal responses to horizont tion should change very little if the tilted out of its plane by small angles the amplitudes should drop rapidly to the tilt angle approaches 90° The corr ing null point at 90° can be used to iden canal driving a given unit in the ve *nerve (Estes et al* 1975 Blanks & *1976) Null point* or better null are mination would be a very insensitive fication technique in the avian cen where the canal plane is encoded in fr terns much more precisely Contr hancing circuitry in the brain is probab to achieve this precision For example vergence of inhibitory vertical and ex horizontal canal input could conceiva *duce the observed* effect This pr

d be difficult to reconcile with the fact very little convergence between electri stimulated canals could be seen in the n s cerebellar cortex (Wilson et al , 1974, e et al , in preparation) Nevertheless, possibility of canal interaction producing nced plane specificity has to be investi- d Of course other plane sharpening cir- y is conceivable It is, finally, still pos- (although not probable) that plane spe- ity greater than given by a cosine func- is available in primary afferents Thresh- measurements to angular acceleration for iary canal afferents in the dogfish indicated threshold differences as long as the la- canal was within 15°–20° of its plane wenstein & Sand, 1940) This "latitude e (Lowenstein, 1974) was greater for ver- l canals than for the horizontal canal ond these ranges thresholds rise steeply se observations might suggest a deviation n the cosine law, they do, however, not ve such unexpected behaviour of primary al afferents because

1) one cosine function can only represent plane If the canal is bent to represent eral planes (as vertical canals in many cies do) the sum of several cosine func- s should describe firing patterns

2) threshold measurements are poor para- ters to assess response magnitude since ob ved thresholds for semicircular canal af- ents always depend upon the sensitivity of recording equipment employed

t can certainly not be assumed that the few t order neurons tested as to this question resent the total range of the semicircular al afferent population and it is interesting note in this context that efferent fibers posed to be inhibitory on afferents, are der influence of converging canal and/or lith activity (Blanks & Precht 1976)

The movement plane correspondence be- een vestibular and neck afferents is striking e identity of neck receptors involved is not ar Joint receptors alone are probably not sponsible, since neck muscle receptors have been shown to converge with vestibular input in the posterior cerebellum (Schwarz et al , in press) It is quite possible that even other re- ceptor types contribute Although all possible care was taken to restrict the stimulation to neck afferents, our paradigm does not exclude excitation of receptors located elsewhere However, if other receptors played a major role, the observed matching of plane spe- cificity with horizontal canal input would only underscore the main conclusion of this study one important aspect of sensory representa tion in the cerebellar cortex is movement direction and plane

This aspect of the sensory projection seems, however, more readily available at the cortical input station than at the (more frequently studied) Purkinje cell output channels Most of the described unitary patterns were found in the granule cell layer and are therefore probably unitary spikes from either granule cells or mossy fibers This does not reflect the distribution of extracellular unitary spikes recorded In fact most spikes were recorded in the Purkinje cell layer where they are much easier to isolate, these did however not yield the desired responses The reason for this preference for granule cell layer response might be the anaesthesia or the immobilisation of the animal

It may seem surprising that the vestibulo cerebellum, so labelled by virtue of its direct connection to the labyrinth (Brodal & Hovik, 1964, Carpenter et al , 1972, Schwarz & Schwarz, in preparation), is not more involved in head rotation than other regions Portions of the uvula on the other hand, not receiving direct vestibular fibers, appear to receive more head rotation information than other cortical portions These aspects of our study should be compared with Haines (1975) description of a rather extensive connection between the pos- terior cerebellar vermis and the vestibular nu clei in primates

An interesting question is, whether the direction of a certain movement is represented in a defined topographical area of the cere

bellar cortex. The great expanse of cortex receiving direction and plane specific information about horizontal head movements tends to argue against this assumption It should however, be realized that head movements in pigeons represent a greater range of motor behaviour than in man: in addition to head rotations performed in a context analogous to human motor behaviour, the pigeon's head is rotated as response of the major vestibulo-visual reflexes, visual tracking reflexes, grasping movements and possibly other patterns. Thus different cerebellar regions, each responsible for the control of a separate pattern, might receive a distinct plane and direction specific input about head rotation.

ZUSAMMENFASSUNG

Neurone im posterioren Kleinhirn einschließlich des Vestibulocerebellum, die auf Rotation in der Ebene des horizontalen Bogenganges antworten, reagieren spezifischer fur die Kanalebene als dies fur primäre Afferenzen bekannt ist Dies zeigt sich in einer Verminderung der Antwortsamplitude, wenn die Drehebene aus der des Kanals geneigt wird, diese Verminderung ist ausgeprägter, als es der bekannten Cosinusfunktion entrechen würde Von 96 solchen Neuronen antworteten [illegible]auch auf Lateralbewegungen um Halsgelenke, nur drei [illegible]eurone reagierten auch auf Hals-Rollbewegungen Neurone mit vestibulo zervikaler Konvergenz fanden sich in Lobulus VI bis X, wobei sich die meisten dieser Zellen in der Uvula und sehr wenige im Nodulus fanden

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VIRAL CULTURE AND ELECTRON MICROSCOPY OF GANGLION CELLS IN MENIERE'S DISEASE AND BELL'S PALSY

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ract Specimens of Scarpa s ganglion during vestib- neurectomy were obtained in 6 cases of Meniere s se and specimens of geniculate ganglion in 2 cases tal facial nerve decompression and studied by tissue re methods for detection of possible herpes and cyto- lovirus infection In electron microscopy inclusions ie form of interwoven yarn like structures coarse egates of chromatin and light nuclear bodies were d in several vestibular ganglion cells No typical es virus virions could be demonstrated The cultures iruses all proved finally negative At present there proof that viruses are present in Scarpa s or genic ganglions but the possibility remains that the in ion bod es observed might be viruses inactivated in the ganglion cells

as long been known that activation of her- simplex virus frequently occurs following eminal root section (Carton & Kilbourne, 2) Ellison et al (1959) found evidence of urrent herpes infection in 18 in a series of such patients and virus was isolated from peripheral lesion but not from two samples Gasserian ganglion All patients possessed ibodies against herpes virus but there was difference in antibody levels in those pa its who developed herpes and those who not

t is generally accepted that virus travels itripetally along the Schwann cells in the isory nerves, usually invading the Gas- ian ganglionic cell nuclei where the non ective core of the virus may remain in a la it stage On activation, new infective par les may be synthesized, released into cyto- ism and carried by means of the axonal flow the epithelial surface where the vesicles are ain produced (Paine, 1964)

Recovery of the herpes virus from the Gas rian ganglion has now been successfully hieved First Bastian et al (1972) isolated herpes virus from 2 out of 23 trigeminal gan glia obtained at autopsy, viral cytopathic ef- fects being evident after 3 weeks Baringer & Swoveland (1973) were more successful isolat ing herpes virus from 6 out of 7 ganglions re moved less than 12 h after death and main tained in culture for 10 to 45 days Baringer (1974) also succeeded in culturing the virus from sacral ganglions innervating genitalia

The concept of herpes virus infection of neural tissue has been extended to Bell s pal sy, particularly as in 25% of the cases there are signs of cranial nerve polyneuritis in the form of transitory hypesthesia of the tri geminal nerve and in 43% involvement of the vestibular system (Adour et al , 1975) In 1972 McCormick put forward a hypoth esis that a large percentage of cases of Bell s palsy could be caused by herpes virus taking up residence in the peripheral nerve cell axon where it was protected from neutralizing anti body and sensitized mononuclear immuno- cytes Infectious virus particles might be pres ent only during the first few days of paralysis and specimens for culture should be obtained early

In a recent study on vestibular ganglion (Ylikoski et al 1977) structures resembling in- clusion bodies were found in the nuclei of sev- eral ganglion cells in two patients with Me- niere s disease This prompted us to try to isolate viruses from vestibular and facial nerve ganglia which became surgically accessible at vestibular neurectomy or facial nerve de- compression

This work was supported by a grant from Signe and Ane Gyllenberg Foundation

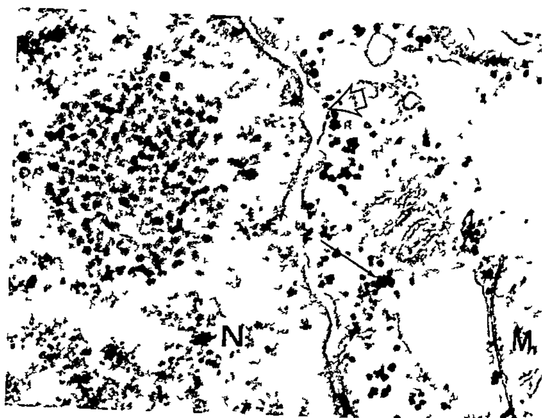


Fig 1 On the left a structure resembl ng interwoven yarn ins de the nucleus of a vest bular gangl on cell The nuclear membranes (*arro v*) are seen n the m ddle of the fgure and on the right the cytoplasm shows SE nbosomes glycogen part cles (*h n arrow*) m (*M*) and a lysosome (*L*) ×85 000

MATERIAL AND METHODS

‹ patients with Meniere s disease one pa tient with Bell s palsy and one patient who was originally thought to have Bell s palsy but had a small Schwannoma of the geniculate ganglion are included in this study Specimens of Scarpa s ganglion and of geniculate ganglion were removed for virus culture and ultrastruc tural evaluation

For virus culture the tissue specimens were transferred directly from the ear to culture medium (RPMI 1640+20% calf serum +1% glutamine + 200 U/ml penicillin + 200 μg/ml streptomycin)

The tissue specimens were minced into pieces and left for absorption on VERO (mon key kidney) and HES (human embryonic skin) cells for 1 h at +37°C in Falcon bottles

Two growth media were used (1) RPMI 1640+20% fetal calf serum + 1% glutamine + 200 U/ml penicillin + 200 μg/ml streptomycin (VERO) (2) MEM + 2% newborn calf s 1% glutamine + 200 U/ml pen cill costatin (HES) The growth med u changed twice a week and cells were p after 2 weeks

After 4 weeks 5 iodo ? deoxyurid ne ml was added for activat on of virus The FA test was performed as f Cells from Falcon bottles with pos t v pathogenic effect (CPE) were detache on glass slides and fixed in acetone The stained for cytomegalo- and herpes v using the indirect technique

For cytomegalovirus hyperimmune serum was used in the first step and fl cein conjugated antihuman globul n r second step (National Bacteriological Stockholm)

For herpes virus anti HSV_1 and globulins rabbit (DAKO immunogl Copenhagen) were used in the first step a the second step fluorescein conjugated

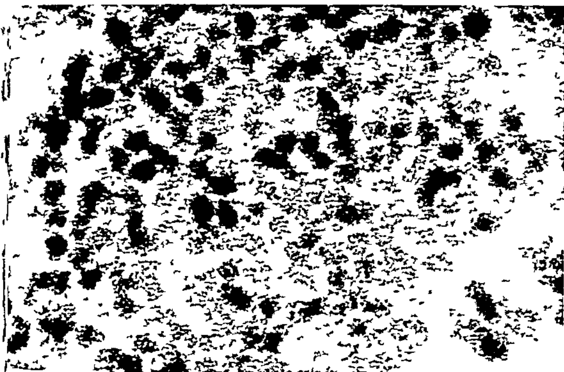


Fig 2 Higher magnification of the yarn like structure in Fig 1 The thickness of the yarn is 20–25 nm and the yarn appears homogeneous without peripheral rims and lighter centre ×?10 000

abbit immunoglobulin sheep (Wellcome Reagents Ltd Beckenham Kent England)

For electron microscopy the nerve specimens were immediately fixed in 0 1 M phosphate buffered 3% glutaraldehyde solution at °C for 2–8 hours stored in sucrose post fixed in 1% osmium tetroxide and embedded in Epon One micron thick sections were stained with methylene or toluidine blue O and studied under the light microscope Sections for electron microscopy were stained with uranyl acetate and lead citrate and studied with JEM 100S or Hitachi HS 7S electron microscopes

Paired samples from each patient were obtained and complement fixation antibody titres determined for viruses in the herpes group influenza para influenza parotitis morbilli neo- and coxsackie viruses Also titres for ornithosis mycoplasma pneumoniae and toxoplasmosis were mostly included None of the patients had any acute viral disease around the time of the surgery

RESULTS

Viral cultures

In 2 patients with Meniere s disease a cytopathogenic effect was evident after 1 month of culture in HES cells The morphologically changed cells formed foci in the cultures and the CPE resembled that of cytomegalovirus Electron microscopy displayed no virus The FA test for cytomegalo- and herpes viruses proved negative The supernatants of the positive cultures were further transferred onto new HES cultures yielding no CPE however

It was suggested that the apparent CPE in the HES cultures was due to proliferation of ganglion cells from the biopsies

Electron microscopy

Three kinds of inclusions were found in the nuclei of the vestibular ganglion cells

1 Structures resembling interwoven yarn (Figs 1 and 2) in small round aggregates Each



Fig 3 On the left the peripheral parts of the nucleus in a vestibular ganglion cell show coarse aggregates of chromatin among lighter chromatin material The appearance of the complex might indicate degradation or formation of the interwoven yarn like structures in Figs 1 and 2 On the right the cytoplasm is without any pathological changes *N*=nucleus *M*=mitochondria ×40 000

aggregate was composed of 20–25 nm thick 'yarn (3 dimensional impression) which in many places appeared contiguous and suggested that the aggregates could be composed of a single long structure Cross sections of the yarn were seen in places These were of homogeneous appearance and did not show a darker periphery (Fig 2)

2 Coarse aggregates of chromatin (Figs 3 and 4) At the periphery of the nuclei 20–60 nm particles of dark chromatin material could be seen Between the coarse particles there was lighter chromatin material Occasionally these coarse particles were seen at the nucleolus (Fig 4)

3 Light nuclear bodies (Fig 5) At low magnification these were seen as light areas in the granular chromatin of the nucleus Higher magnification displayed hazy fibrillar structures arranged around a few interchromatin granules The diameter of these bodies was 0 8–1 0 μm (800–1 000 nm)

DISCUSSION

The interwoven yarn like structures seen in the nuclei of vestibular ganglion cells share certain morphological features with microtubular inclusions i e aggregates of tubular structures with a diameter of 22 nm These inclusions are seen inside the endoplasmic reticulum (Uzman et al 1971 Schaff et al 1973 Barnety et al , 1974) and have a more distinctly tubular appearance than the structures we found in the nuclei Originally it was believed that the microtubular inclusions were aggregated myxoviruses or paramyxoviruses Later studies however, have not been able to prove their specificity (Uzman et al 1971 Barnety et al , 1974) or show that they contain

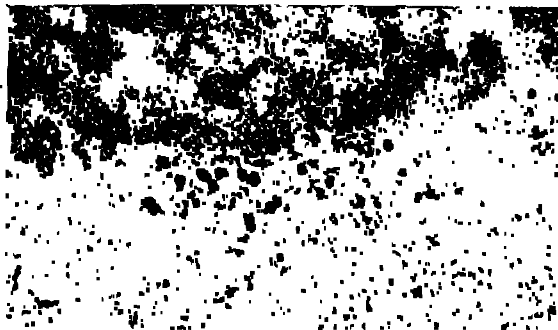


Fig 4 Coarse aggregates of chromatin at the nucleolus (above) Note the homogeneous appearance of the particles They do not show peripheral membrane ×80 000

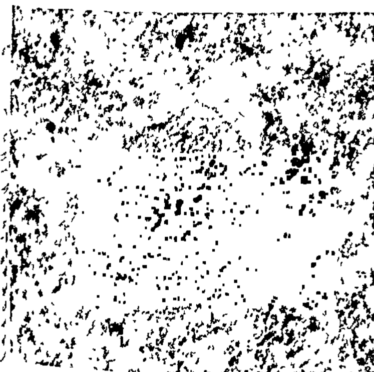


Fig 5 Light nuclear body in the nucleus Note the delicately fibrillar periphery and the central interchromatin granules ×80 000

nucleic acid (Schaff et al, 1973) In SSPE (subacute sclerosing panencephalitis), nucleocapsids which have a diameter of about 20 nm can occur in the nucleus (Raine et al, 1973, Iwasaki & Koprowski, 1974) However, also these have a more distinctly tubular appearance than the structures we found in the nucleus So far there are no reports on studies that have been able to link corresponding structures with active herpes or cytomegalo virus infection Of course, this also applies to the coarse aggregates of chromatin which do not show a constant diameter, suggesting that they are not viruses or parts of viruses

Light nuclear bodies were first described by de The et al (1960) and Hinglais Guillaud et al (1961) These structures have attracted the attention of several investigators (e g Weber & Frommes, 1963, Bouteille et al, 1967, Krishan et al, 1967) and they are found in numerous tumours, virus infected cells and also under normal conditions In no way do they appear to be specific to any virus Nuclear bodies have been reported in studies in olving viral activity (Dubois Dalcq et al, 74) and Maiztegui et al (1975) showed that i Argentine hemorrhagic fever, fluorescein labelled ascitic fluid, hyperimmune to Junin virus, was bound to these nuclear inclusions One study of multiple sclerosis reported similar structures, which also contained nucleocapsids (Iwasaki eta l, 1973) There is much to be said for the idea that the light nuclear bodies are structures linked with normal nuclear activity and one of the authors has found them in kidney biopsies in numerous kidney diseases (e g Runeberg et al, 1975)

However, it is possible that these structures could be activated in viral infections and under those circumstances they might produce viral components

Our study could not link the nuclear structures with herpes or cytomegaloviruses They might be linked with other viruses, even though earlier reports suggest that one of the inclusions can be found under normal conditions It should also be borne in mind that all the possible interrelationships between viruses and nerve cells are not known This is why we do not want to exclude the possibility that the structures observed could be caused by or could be linked with viruses inactive inside ganglion cells (Waterson & Almeida 1969)

ZUSAMMENFASSUNG

Spezimens aus Ganglion Scarpa und Ganglion ge wurden in 6 Fallen von Menierescher Krankheit Fallen von Facialisparalyse wahrend der Neurektomie bzw totale Facialisdekompression nommen und mit Gewebekulturmethoden für Herpes Zytomegaloviren untersucht In elektronenmikroskopischen Untersuchungen der vestibularen Ganglien den drei Typen von Inklusionen gewundete fadenliche Strukturen grobe Chromatinaggregate und nukleare Korper gefunden Keine typischen Herpes oder Zytomegalovirus Virionen wurden beobachtet gibt keinen Beweis uber das Faktum daß es Viren in Ganglion Scarpa oder Ganglion geniculi aber es ist moglich daß die Inklusionkörper Viren innerhalb der Ganglionzellen reprasentieren.

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SIALOCHEMICAL EXAMINATIONS IN NON-TUMOROUS PAROTID ENLARGEMENTS

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Abstract Secretion rates and the composition of isolated [illegible]

of the results indicated that sialochemical examination may provide a valuable help in the differential diagnosis of parotid diseases characterized by swelling of the gland Chronic parotitis is characterized by a significantly decreased flow rate extremely high sodium and protein concentration and lack of secretory response to stimulation In Sjogren s syndrome the low flow rate was found to be associated with a decreased potassium secretion rate as well as decreased flow rate and decreased sodium concentration after stimulation In sialosis where flow rates display a considerable individual variation a significant increase in potassium secretion rate could be revealed

Enlargement and hypofunction is a common symptom of many diseases of the parotid gland Tumours may often be excluded by means of clinical history, palpation and sialography On the other hand, correct diagnosis may be hindered by non-tumorous swellings of the salivary glands

The chronic recurrent parotid swellings most frequently encountered in clinical practice may be classified into three groups, namely chronic recurrent parotitis, rheumatoid sialadenitis (Sjogren's syndrome) and sialosis Although distinction of these enlargements is important both for therapy and prognosis, clinical history and examination are often insufficient to make this distinction Furthermore, sialographic examination often fails to establish a diagnosis in many cases, since radiographic alterations are frequently missing or are not specific for the particular pathological states (Diamant & Fenske, 1959 Rauch & Gorlin, 1970 Chisholm et al 1971, Chisholm & Mason, 1973)

There have been attempts for a long time to draw diagnostic conclusions from the changes in exocrine function of the gland The [illegible] metric and sialochemical changes observed in various non tumorous parotid diseases have been surveyed by Rauch (Rauch 1959 Rauch & Gorlin, 1970) Albeit some parotid diseases are associated with characteristic changes in secretion rate or in the salivary concentration of some ions and protein estimation of salivary gland function does not belong to [illegible] diagnostic means This may be accounted for both by the tedious character of these examinations and the frequent contradictions of literary data These conflicting observations may be explained by differences in saliva collection methods as well as the non-standardized physiological and environmental factors affecting the function of salivary glands

In our previous reports (Benedek Spät [illegible] a, b) an account has been given on [illegible] flow rate and parotid salivary composition in the resting state and after stimulation as observed in healthy university students [illegible] studies on parotid function in Sjögren's syndrome have revealed that, besides a decreased secretion rate and increased protein concentration of saliva, the syndrome is characterized by a diminished responsiveness to stimulation (Benedek-Spät et al , 1979)

The present work was designed to [illegible] changes in flow rate or composition of [illegible]

e I *Distribution of the examined subjects according to diagnosis, sex and age*

	Number of patients	Sex		Age (years)	Histological verification
		Male	Female		
·ol	17	5	12	51±3	–
n parotitis	6	2	4	52±4	–
en s syndrome	21	4	17	49±3	8
sis	24	10	14	50±2	3

va in three forms of parotid swelling, onic recurrent parotitis, rheumatoid siala- itis and sialosis

METHODS

· examinations reported here have been ried out in 68 subjects Diagnosis, sex and of the patients are shown in Table I For itrol purposes we examined subjects who not suffer from any systemic or salivary nd disease and did not take drugs

Diagnosis of chronic recurrent parotitis has ·n based on clinical history as well as on tenological and sialographical examina- ns

Diagnosis of Sjogren's syndrome has been ablished on the characteristic clinical signs d laboratory data and, in about one third of cases on histological examination Some tients with verified Sjogren's syndrome had swollen parotids at the time of sialochemi- l examination Considering, however, that difference in respect of parotid function s been observed between these patients and ose having parotid swelling, all these data re pooled into one group

Table II shows the diseases associated with d usually preceding the bilateral parotid elling in our sialotic patients Sialography iled to reveal any significant alteration in ese patients Histological examination veri d the diagnosis in three cases

Saliva was collected in the fasting state, tween 8 00 and 9 00 a m by means of a n polyethylene tube introduced into one or oth parotid ducts Details of the technique · · previously described (Benedek Spät, 1973 *a*) Unstimulated saliva was collected for 10 or 20 min, depending on the flow rate After an interval of several minutes the secre tion was stimulated by a 2% solution of citric acid, of which two drops were applied every half minute on the upper surface of the tongue Stimulated saliva was collected for 3 to 5 minutes No chemical analysis could be undertaken when the volume of saliva was below 50 μl

Secretion rate, both before and after stimu- lation, was measured and the samples were analysed for sodium, potassium and protein concentration by methods previously detailed (Benedek Spät, 1973 *a*) Amylase activity was estimated with Phadebas Amylase Test (Phar macia)

Mean values and standard error of the mean (S E) were calculated With regard to the fact that only unilateral examinations were undertaken in some patients, while bil ateral examinations were executed in others, standard methods used for statistical calcula- tions have been correspondingly modified

Table II *Associated diseases of 24 sialotic patients*

Diabetes mellitus	9
Hypothyroidism	3
Oligomenorrhea or dysmenorrhea	5
Long term steroid treatment	2
Chronic alcoholism	2
Chron hepatitis	1
Iron deficiency	1
Bronchial asthma	2
High blood pressure	6
Neurotic complaints	9
Rheumatoid arthritis	2

diseases examined by us Cannulation of the gland helped the diagnostic work also in cases where measurable amounts of saliva could not be collected, as it provided a sample for bacteriological examination

Swollen parotids, generally on both sides, may often be encountered in Sjogren's syndrome, a kind of autoimmune disease Such a swelling presents no diagnostic problem when the characteristic clinical symptoms and laboratory changes are manifest (Bunim et al , 1964, Rauch, 1966, Whaley et al , 1969, Cummings et al , 1971) Not infrequently, however, a patient will complain of a swollen gland or hyposecretion when the characteristic clinical symptoms have not yet developed, and pathological changes cannot yet be detected in the sialogram (Chisholm et al , 1971, Chisholm & Mason, 1973, Ericson, 1974) *On the basis of a few reports dealing with the* sialochemical changes in Sjogren's syndrome it is accepted that, in common with other infectious inflammations, hyposecretion also in this disease is accompanied by a high sodium concentration (Rauch, 1959, Mandel & urmash, 1973, 1976) Our ealier study on this disease (Benedek-Spat et al , 1975) indicated that the secretion rate might depend on the progression of the disease The sodium concentration of the saliva depended on the flow rate we observed high sodium values in cases only where the flow rate was below 0 01 ml/min Normal sodium concentration values indicate that the sodium reabsorbing capacity of the duct epithelial cells is not severely impaired in rheumatic inflammation *The extremely high sodium concentration values* found in cases of very low flow rate may be accounted for by sodium re equilibration in the more distal region of the excretory duct (cf Schneyer et al 1972 Young 1973) The present examinations confirm these findings on the basis of a larger number of observations Despite some high sodium values found in cases of extreme hyposecretion the mean sodium concentration did not differ significantly from the control Characteristically for Sjögren's syndrome, we found an increased concentration of protein and increased [illegible]lase activity, besides *the well-known decrease* in secretion rate For distinction between Sjögren's syndrome and sialosis the potassium secretion rate below 1 μEq/min in the S[illegible] group may be of great importance (Fig 3)

Sialosis is the term used to describe a non inflammatory, non neoplastic recurrent bilateral swelling of the salivary glands. The majority of reported cases have been related to hormonal or neural disorders malnutrition cirrhosis of the liver, and chronic alcoholism (Rauch, 1959, Seifert, 1971, Seifert & Do[illegible] 1975, Donath, 1976)

The mean secretion rates in our sialotic patients were significantly higher than in controls This increase in secretion rate however, is of no diagnostic significance since *the range of individual observations* extends from very low to very high values (Fig [illegible] Sialosis is generally thought to be characterized by low flow rates (Rauch 1956 19[illegible] Nonetheless, high values for secretion r[illegible] were reported in sialotic cases of neur[illegible] (Rauch, 1959) or alcoholic origin (Abelson et al , 1976) as well as in the early stage of disease (Diamant, 1974) In agreement with these observations, 5 of our 8 highest va[illegible] were obtained from chronic alcoholic or sialotic patients Nevertheless even disregarding these high values, we do not regard a[illegible] creased flow rate as characteristic for sia[illegible] Conflicting opinions in this respect may [illegible] attributed to the dependence of flow rate [illegible] the progression of the disease or the admini*stration of different* drugs because of an as[illegible] ciated disease

An increased potassium concentration [illegible] the parotid saliva of sialotic patients was [illegible] ported by Rauch (Rauch 1956 1960) but [illegible] were unable to observe such an increase [illegible] discrepancy between these results may [illegible] haps be attributed to the same factors as [illegible] cussed for the flow rate More important however, is the *observation* that the p[illegible] sium secretion rate is much higher tha[illegible]

gren's syndrome (Fig 2) Consequently parameter would seem to be a useful ns for differential diagnosis

stimulation with citric acid is followed by a racteristic increase in secretion rate and in ium concentration of the saliva in healthy jects Stimulation failed to augment the retion rate in chronic recurrent parotitis was painful in some patients While sia- ic patients responded to stimulation in a nner comparable to healthy controls, the ulated flow rate in Sjogren's syndrome s significantly smaller than in controls, as l as sialotic patients The observation of flow rate in Sjögren's syndrome is in eement with the reports of Sewards et al 66) and Chisholm & Mason (1973) While low flow rate does not mean a decreased ponsiveness to stimulation (the ratio of nulated to unstimulated flow rate is com able to that in healthy subjects) there is a luction or even a lack of response in re- ct of sodium concentration Since the so- im response is maintained in sialosis, ex- ination of sodium concentration before and er stimulation may, again, be of diagnostic portance

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ZUSAMMENFASSUNG

s wurde bei 51 an chronischer rezidivierender Parotitis lose oder Sjogren-Syndrom leidenden Patienten und i 17 gesunden Kontrollpersonen das Maß der isolierten rotis-Speichelsekretion und die Zusammensetzung der rotis-Speichel untersucht Die Bewertung der Ergeb- ise ergab daß die sialochemische Untersuchung in der klerung der Speicheldrusengeschwulste verschiedenen rsprungs wertvolle Hilfe leisten kann Die chronische rotitis ist von der signifikant verminderten Sekretion n der äußerst hohen Natrium und Protein Konzentra n ferner vom Mangel der auf Stimulierung gegebenen kretorischen Antwort charakterisiert Im Sjogren-Syn om war die niedere Speichelsekretion von verminder a Kalium-Sekretionswerten begleitet und nach Stimu tion war das Maß der Sekretion und die Natrium Kon ntration signifikant kleiner als bei den Kontrollwerten. In Sialose war außer der individuellen Schwankung der Sekretion die signifikante Zunahme der Kaliumsekretion charakteristisch

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THE MUCOCILIARY ACTIVITY OF THE UPPER RESPIRATORY TRACT

II A Method for In Vivo Studies on Maxillary Sinus Mucosa of Animals and Human Beings

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tract A method for photoelectric in vivo recordings the mucociliary activity of the mucosa of the upper p ratory tract is described and tested in model experi nts In vivo recordings from the maxillary sinus mu sa of rabbits were easy to analyse and the method is ful for further experimental research on animals amples of in vivo recordings from the human maxillary us during Caldwell Luc operations are also presented ese results are compared with subsequent in vitro re rd ngs of biopsy material

veral authors have made human in vivo stu es of mucociliary transportation by deposit g tracer substances in the nose (Hilding, 32, Ewert, 1965, Proctor & Andersen, 1976), aranasal sinuses (Yates, 1924, Hilding, 1944, lesserklinger, 1966) and the middle ear (Sade, 67)

In pathological conditions retardation and agnation of secretions have been observed hether this is due to changes in the amount nd tenacity of secretions or impaired function f cilia is not easy to evaluate although vig rous activity of cilia has been observed in sin ses even in the presence of 'overwhelming nfection of long standing' (Proetz, 1933)

The pattern of cilia movements within the mucus layer can be assessed from periodic hanges in light reflections observed on the surface of the mucous membrane through an operating microscope (Frenckner & Richtner 1939) Recording methods allowing direct eva luation of the frequency of this surface activity have been improved during the last decade e g by Håkansson & Toremalm (1965) Guillerm et al (1965), Chevance & Lennon (1970) and Mercke et al (1974) Use of these methods has hitherto been limited to experimental in vitro studies

Simplified equipment for recording local mucociliary activity has been developed and technical data as well as preliminary in vitro recordings from human material have already been described (Reimer et al 1977)

The aim of this paper is to report on the possibilities and limitations of the method in use on the maxillary sinus mucosa of rabbits and human beings in vivo

MATERIAL AND METHODS

The mucociliary activity is recorded indirectly via the variations of surface light reflections brought about by the movements of cilia The reflected light is focused by an operating microscope (Zeiss OPMI I), transformed to electrical signals by a phototransistor (Type FPF 139) and recorded by an ink writer (elema Schonander Mingograph 34)

An oscilloscope facilitates focusing on a reflecting area of the mucosa A bandpass filter (3 30 Hz) supresses high frequency interference The recordings represent the light reflections from a circular mucosal area about 0 1 mm in diameter The method and equip-

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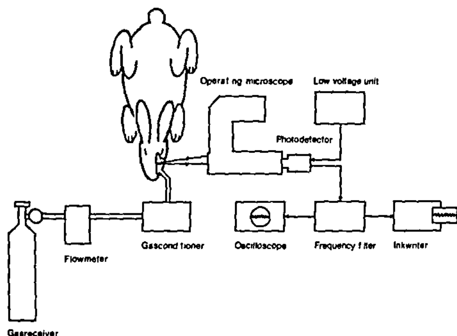


Fig 1 C
for in vivo experim
rabbit maxillary sin
text)

ment has been described in detail in a previous paper (Reimer et al , 1977)

Model experiments

he frequency and amplifying characteristics the equipment were tested experimentally by recording from the illuminated surface of a drop of oil spread over a piece of plastic placed on a vibrating loudspeaker membrane In a series of experiments tracheal specimens from rabbits with active ciliary movements were recorded from when absolutely still, and when moved by the loudspeaker membrane vibrating at different frequencies and amplitudes

By altering the temperature of the specimen different mucociliary wave frequencies we achieved The induced vibrations were a justed to a recorded amplitude of 1-2 t that of the mucociliary recording

Animal recordings in vivo

Rabbits (weight 2–4 kg) were anaesthet with Nembutal® intravenously (40 mg/kg i tially, thereafter approximately 20 mg/kg h hole of about 5×5 mm was made in the n ridge and the maxillary sinus was expo The medial wall was illuminated and a ref ing surface of about 2–4 mm² was focu The mechanical stability of the rabbit h was assured by a special holder The h could be moved in any direction by mean micrometer screws Conditioned air was troduced to the sinus via an additional t The experimental set up is shown in Fi and examples of recordings in Fig 5 O to accumulations of mucus or to unavoid movements of the equipment during th periments, minor adjustments of focus w sometimes necessary The range of freq cies was studied on three reflecting areas exposing a greater part of the medial wa the sinus

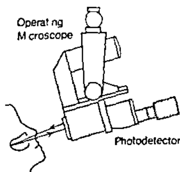


Fig 2 Operation situation for clinical in vivo recordings

ical recordings in vivo

patients were under general anaesthesia (Halothane®) and intubated orotracheally The head rested in a circular sandbag on an ordinary operating table The clinical application is illustrated in Fig 2 Since the original method was designed for an exact replica of an adult maxillary sinus (Reimer et al, 1977), the microscope and the ancillary equipment could be readily adapted for in vivo studies in the operating theatre The microscope was enclosed in a sterile bag The phototransistor is small and light that it can easily replace one of the microscope eyepieces without disturbing vision through the other After careful haemostasis the antrum was opened *ad modum* Caldwell Luc With a magnification of about ×, a light reflecting area on the posterior wall could be kept in focus and the mucociliary activity recorded Accumulation of mucus and blood had to be cautiously sucked away occasionally After the in vivo recordings the actual part of sinus mucosa was carefully extirpated and within about 20 min placed in an experimental chamber with the size and configuration of an adult maxillary sinus Recordings were commenced 10–15 min later at an ambient temperature of 37°C and relative air humidity of >80%

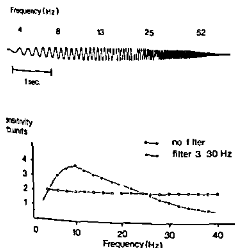


Fig 3 Recording from a light reflecting surface on a loudspeaker membrane vibrating with constant amplitude from 3 to 80 Hz (*upper*) The amplitude at frequencies above 30 Hz is attenuated by a filter Relative sensitivity at different frequencies tested in the same manner with and without the filter (*lower*)

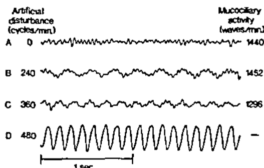


Fig 4 Recordings from a tracheal specimen placed on a vibrating loudspeaker membrane The added movements are easily recognized (*B* and *C*) When the amplitude of the induced vibrations is too large the mucociliary waves are concealed (*D*)

Control recordings

Possible sources of disturbance are movements by the patient (muscular, cardiovascular and respiratory trembling) and by the operating microscope The mechanical disturbances in vivo were checked by recording the light reflected from a piece of aluminium foil placed on the forehead of the patient The mechanical stability in vitro was tested in the same manner from a piece of aluminium foil placed in the bottom of the experimental chamber

RESULTS

Model experiments

A white piece of plastic, covered with a drop of oily liquid, offers a smooth surface without irregularities The frequency of the recording follows the frequency of the induced vibrations exactly (Fig 3 upper) The attenuated amplitude at frequencies above 30 Hz is due to the filter The effect of the filter on recordings from a surface vibrated with constant amplitude at different frequencies is shown in Fig 3 lower

Recordings from a mucous membrane with active cilia are seen in Fig 4 When the mu

Table I *Frequencies (waves/min) based on recordings of 10 sec duration*

Expt no	Time (minutes) 0	1	2	3	4
No artificial disturbance					
1	1 398	1 380	1 344	1 350	1 350
2	1 410	1 404	1 416	1 422	1 422
	Artificial disturbance (cycles/min)				
	0	240	360	480	0
3	720	666	720	882	720
4	858	954	954	846	801
5	990	1 032	948	870	1 008
6	1 284	1 266	1 254	1 170	1 296
7	1 446	1 404	1 350	1 320	1 380

cous membrane is vibrated at 240 to 360 Hz, an extra undulation is seen in the recording The frequency of the mucociliary waves can still be calculated but an error is introduced (Fig 4C) If the amplitude of the extraneous movement is too great (Fig 4D), the mucociliary waves will ultimately be swamped and e recordings no longer represent the movements of cilia The results of the recordings at different mucociliary wave frequencies and a disturbance amplitude of 1–2 times that of

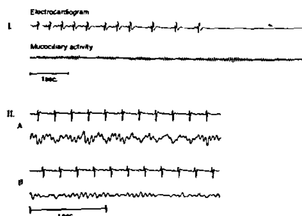
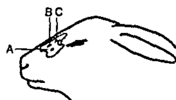


Fig 5 Recordings from the maxillary sinus of rabbits I A regular wave movement and amplitude modulation during the final heart beats after an overdose of Nembutal® II Recording of mucociliary wave movements and



Area	Point	Frequency (waves/min)
A	1	1416
	2	1422
	3	1452
B	1	1437
	2	1374
	3	1434
C	1	1182
	2	1188
	3	1296
	4	1308

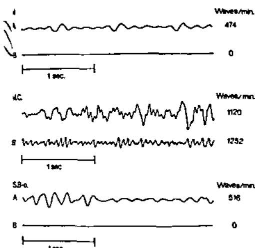
Fig 6 The range of frequencies on three light areas on the medial wall of a rabbit maxillary sinus is outlined in natural size related to the hea

the mucociliary wave recording is g Table I The error introduced by such turbance gives no remarkable deviatio the expected frequency

Animal recordings in vivo

In Fig 5, I, a recording during the fin beats from an animal killed with an ov of Nembutal® is shown There was mediate change in character or frequ the mucociliary light reflections In so bits a low frequency undulation of the ings was seen (Fig 5, II A) which disap after a minor adjustment of the rabb (Fig 5, II B) The simultaneous electr gram shows that the cardiac rhythm this disturbance, which is thus proba rived from pulsations of the vascular b disturbance is easily recognized and j the model recordings (Fig 4B C) d interfere with the calculation of the m ary activity

The range of mucociliary wave freq from different reflecting parts in one shown in Fig 6 It is seen that the rang one reflecting area is small, but grea ferences can be seen between differen



7 *Control* Recordings from a piece of aluminium foil he forehead of a patient during apnoe (A) and in the illary sinus model (B) M C The mucociliary activity ivo (A) and in vitro (B) in a case of purulent sinusitis text) S B O Recordings from the mucous mem ne in vivo (A) and in vitro (B) in a case with apparently mucociliary activity (see text)

nical recordings in vivo and vitro

ceptable conditions regarding extraneous sturbances were achieved only during ap a Extraneous disturbances are recorded m the aluminium foil on the forehead of a tient (Fig 7, Control, A) A corresponding *vitro* recording shows complete absence of sturbances (Fig 7, Control, B)

Two combined in vivo and in vitro record gs are shown below The first patient, M C, d a long standing purulent sinusitis In vivo ecordings from the posterior wall show a riodic movement, which is disturbed by ir- gular waves of larger amplitude It was pos ble to count the periodic movements during sec of the recording The calculated fre uency was 1 120 waves/min (Fig 7, M C,) In vitro recording from the same part of e mucosa revealed a regular wave movement f 1252 waves/min (Fig 7, M C, B) In the ext case (S B O) a cyst occupied the an um There was no visible mucociliary activ- y on the *posterior wall* The recording from e illuminated mucous membrane (Fig 7, S -O, A) probably only represents movements of the patient and/or the operating microscope since the corresponding in vitro examination confirmed the absence of mucociliary activity (Fig 7, S B -O, B)

DISCUSSION

The importance of experimental and clinical research regarding the local mucociliary activity of the extrathoracic airways has been discussed in previous papers (Mercke et al, 1974, Reimer et al, 1977)

The mucous membrane in these regions constitutes an ideal object for functional studies, since the cilia continue to work for several hours even after removal from the body This activity can therefore be studied in vitro under carefully standardized conditions However, in vivo studies are also needed, for example to find out how far the mucociliary activity is depended on a continuous vascular supply and neural control

The light intensity recorded from an illuminated part of the respiratory mucous membrane can fluctuate due to movements from different origins (Toremalm et al, 1974) During in vivo conditions, cardiovascular and respiratory trembling may disturb the recording of the movements produced by cilia and mucus The influence of extraneous movements has therefore been analysed experimentally

The model experiments showed that mucociliary wave movements can be recorded indirectly even from an object which is not absolutely still, provided the extraneous disturbances are within certain limits related to the mucociliary recordings During animal experiments, mucociliary activity recordings sometimes exhibited irregular low frequency events which coincided with simultaneously recorded cardiac beats This activity varies from place to place in the same sinus, and is probably derived from pulsations in the vicinity of the vessels (Fig 5, II) The recordings from specimens during induced vibration support this hypothesis (Fig 4, B and C)

In vivo recordings from the rabbit maxillary sinus were easy to analyse, and the frequency range of each examined area was small (Fig. 6) These facts make this application of the recording method very useful in experimental research

The recordings from humans had to be obtained during fairly short apnea periods of 20–30 sec, since the head of the patient could not be fixed as effectively as the head of an animal The mechanical stability of the operating microscope fixed on a floorstand is quite good but cannot be compared to the rigid laboratory equipment This is seen in the result of the control recording from a piece of aluminium foil on the forehead of a patient (Fig 7, control, A) In addition, inflammation of the mucous membrane presumably brings about more intense vascular pulsations than in healthy tissues We attribute the irregular low-frequency background activity seen in the in vivo recording of case M C. (Fig 7, M C, A) to these factors The *in vivo* recording from [illegible] other case (Fig 7, S B -O, A) represents [illegible] irrelevant extraneous movements only, [illegible] the corresponding *in vitro* examination revealed no mucociliary activity Inflammatory diseases of the paranasal sinuses and the middle ear are often characterized by retention of secretions It is therefore of great interest to elucidate the functional state of the ciliated epithelium in such disorders

Further combined in vivo and in vitro examinations of the human maxillary sinus, as well as extended functional and morphological studies on other human specimens from the upper respiratory tract, will be reported in a forthcoming paper

ZUSAMMENFASSUNG

Beschreibung einer fotoelektrischen Methode zur Registrierung *in vivo* der mucoziliaren Schleimhautaktivi [illegible]

[illegible] sonders für weitere Tierexperimentelle Forschungszwecke Als Beispiel werden auch *in vivo* Aufzeichnungen von menschlichen *Kieferhöhlen* während der [illegible] nach Caldwell Luc dargestellt Diese [illegible] mit anschliessenden *in vitro* Aufzeichnungen [illegible] siematerial verglichen

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EXPERIMENTAL SURGERY ON THE NOSE

I *Airflow and Goblet-cell Density*

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tract In rabbits the nasal vestibule was surgically oc led on one side The rabbits were observed for vary periods maximum 90 days The mucosa of the septum removed from both sides stained by the PAS alcian whole mount method and the goblet cells were nted The difference in goblet-cell density between the luded and open side of the nose was less than ex ted according to previous reports A completely lished air flow for 3 months caused only some increase lensity, doubling of the air flow some decrease in gob- cell density During the first 16 days there was an in ase in density on the occluded as well as on the open e of the nose This can hardly have been due to al d airflow but must be attributed to an irritative action the operation upon the nasal mucosa

he most important function of the nose is to otect the lower air passages Mucus produc- on and ciliary function are important factors conditioning the air passing through the se Mucus forms in the goblet cells, intra ithelial glands, and in the submucous, tubu- alveolar glands The activity in the submu- ous glands is accelerated by parasympathetic imulation (Ånggaard, 1976) and by oestro- nic substances (Helmi et al , 1975, Mortimer al , 1936) However, studies on the func- onal role of goblet cells in mucus production re lacking and we possess only limited nowledge of the factors which influence their umber, activity, and turnover in the nose

Hilding and his associates (1932, 1970, 1970) arried out pioneer animal experiments for the urpose of elucidating the role of the air flow the morphology of the nasal mucosa By nilateral, surgical occlusion of the nasal ves- ibule they demonstrated in rabbits an increase n goblet cells on the occluded side and meta- plasia of the respiratory epithelium towards stratified squamous epithelium on the open side The interesting studies of Hilding et al were based upon an estimate of the quantita- tive increase in goblet cell density, and they only state the time of this increase

In clinical practice, we often come across differences in the passage of air through the two nostrils without being able to demonstrate any definite macroscopic differences in the mucosa It has also been demonstrated (Proetz, 1953, Masing 1967) that the mucosa on one side of the nose is not uniformly af- fected by the inspired air and that in certain parts of the nose the air flow is stronger than in others Differences in the strength of the air flow constitute a physiological phenomenon Therefore, the mucosal changes that they pos- sibly cause may be imagined to be so subtle that they are difficult to record with reason- able accuracy by a subjective estimate of con ventional histological sections Objective, quantitative studies are needed They can be performed on the epithelial goblet cells by whole-mount methods (Moe, 1955) in which the epithelium is stained by PAS (Moe, 1955), or by PAS alcian blue (Kessing, 1968, Tos, 1970), and the goblet cells are counted on the surface in several, well-defined fields

If the pathophysiology of the nose is to be *better understood, it is important to establish* the extent to which the mucosa is altered by a reduced, abolished, or increased air flow, when this change occurs, and by what factors, other than the air flow, it is influenced



Fig 1 Closed and well adapted right nasal vestibulum (*a*) Frontal (*b*) lateral view

MATERIAL

Rabbits of the type Copenhagen White from Statens Seruminstitut, Copenhagen, were used The rabbits were adult, weighing about 3 kg Body weight was recorded before the operation and at sacrifice, no rabbit had lost ; The material was divided into three , (Table I) 1 Normal rabbits (11) living der the same conditions as the others 2 Anaesthetized rabbits (7) matched to each group of operated rabbits in order to ave the influence of anaesthesia 3 Operated r bits (20) whose right nostril was occluded that respiration was exclusively through t left one

METHOD

All operations were carried out under gene anaesthesia using i v Nembutal Rabbits ha extraordinarily active movements of the

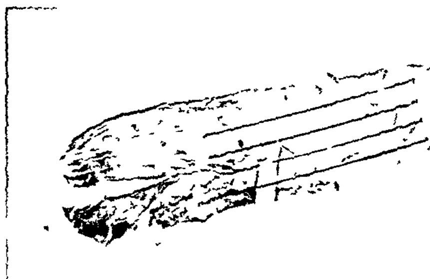


Fig 2 Whole mount preparation of right nasal septum The part on A horizontal biopsy has been taken from the anterior p and a vertical biopsy from the posterior part of respiratory region ×2

ble I *Number of animals in each group Number of rabbits which for different reasons should excluded from the operated material*

e of rvation s)	Normal animals	Anaesthetized animals	Operated animals	Discharged animals
	2			
		1	3	
		1	3	
		1	3	1 catarrhalia 1 right side
			1	
		1	2	
	3	1	2	1 defect closure
	3	1	3	2 catarrhalia 1 left side
	3	1	3	1 catarrhalia
–	11	7	20	

s and with their paws they will try to reove any foreign body in this region (if both strils are occluded, they die of respiratory lure) After testing various surgical proceres, we selected the following A couple of m from the external orifice an oval incision as made in the entire circumference of the stibule The mucosa was undermined, inginated, and the margins from the medial id lateral side sutured with submucous sures of absorbable material The skin was refully closed with silk sutures (Fig 1) Two ctors seem to be of particular importance securing stability of the operation To avoid flammatory reaction the sutures in the muosa must be submucous and must not peneate into the nasal cavity It is very important also to undermine the mucosa in a considerable area, as a taut mucosa over the medial, cartilaginous part of the septum will easily lead to perforation

The rabbits were killed with an overdose of Nembutal i v After sacrifice, the nasal septum was removed *in toto* and fixed in formalin alcohol The entire mucosa on both sides was removed from the underlying, cartilaginous part of the septum, microdissected with removal of the deeper part of the lamina propria, stained with PAS-alcian blue (Tos, 1970), and embedded in a chamber with a solution of anise oil–colophonium (Fig 2)

Goblet cells were counted in a Reichardt projection microscope, magnification 500×, in fields measuring 0 01768 mm² Four equidis-

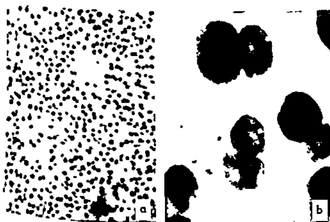


Fig 3 Distribution of goblet cells seen in whole mounts PAS-alcian blue staining (*a*) ×80 (*b*) ×800

Table II *Individual median density of goblet cells (cells/0 01768 mm²) in the right and* [illegible] *of the septum from normal, anaesthetized and operated rabbits*

Observation period (days)	Normal		Anaesthetized		Operated	
					Closed	Open
	Right	Left	Right	Left	Right	Left
0	40	20				
	77	76				
4					47	47
			46	44	59	47
					95	55
6					54	47
			25	66	59	80
					113	84
10					104	45
			84	46		
						81
16					110	83
20					52	18
			34	41		
					76	42
30	32	50				
	55	63	25	86	46	43
	69	76				
60	35	38				
	65	61	78	65	84	
	71	79				
[illegible]	11	23			59	20
	55	44	76	55		
	58	86			64	51
[illegible]	55	61	46	55	62	47
Median	57		50			

tant, horizontal lines were drawn on the slide, and every tenth field along these lines was counted, starting anteriorly at the junction to the vestibule (Fig 2)

RESULTS

Four of the operated rabbits had to be excluded from the quantitative analysis because of catarrhalia and one because of a minor defect in the closure (Table I) Moreover, one right and one left septal mucosa had to be excluded for histotechnical reasons

Morphology On the whole mounts the goblet cells stood out as bluish sharply defined, round to oval spots on a paler bluish-green background (Fig 2) In normal rabbits the size varied only slightly around 6 μm in diameter, but scattered in the epithelium [illegible] were also goblet cells measuring 10-12 μm [illegible] even 20 μm in diameter

The distribution was irregular, man[illegible] itself as differences in the density with[illegible] same counting field, between various [illegible] bouring fields, and between different [illegible] This made for a marked dispersion of [illegible] density for each septum and emphasizes [illegible] the goblet cells in each septum have [illegible] counted in many fields to obtain a rel[illegible] median density, and it also goes to show [illegible] an estimate of the density on the basis of [illegible] tological sections can afford no defi[illegible] pression of the density

It was estimated that the ratio between [illegible] and large cells was the same on the [illegible] and on the patent side, as in normal rab[illegible]

le III *Individual median density of goblet cells (cells/0 01768 mm²) in the 12 anterior and posterior fields of the septum from normal anaesthetized and operated rabbits*

ight L=left Ant =anterior Post =posterior

er on od s)	Normal				Anaesthetized				Operated			
	Ant		Post		Ant		Post		Closed (R)		Open (L)	
	R	L	R	L	R	L	R	L	Ant	Post	Ant	Post
	66	23		14								
	49	58	85	70								
					45		31		93	83	21	50
						57		34	27	52	47	38
									46	71	43	56
					27		37		69	67	63	80
						86		66	125	101	73	82
									84	69	16	53
					39		100		88	99	109	72
						43		75			47	56
									172	94	74	64
					35		43		65	67	50	38
						43		45	102	53	12	53
	94	53	60	63	20		46		93	31	58	39
	14	27	65	48		124		68				
	69	31	18	106								
	83	90	61	72	100		86		94	65		
	52	72	79	59		42		73				
	32			45								
	67	40	38	44	46		77		64	62	23	24
	49	112	36	44		53		60	60	55	66	29
	64		32	42								
dian	64	53	60	48	39	53	46	66	86	67	49	53
ed an		56		54		44		63		77		51

Density of Goblet Cells

ormal material The individual median den ty of each septum, based upon counting bout 30 fields, varied somewhat from nose o nose (Table II) from 11 to 86 cells/field here was no significant difference in inter idividual median density between the right nd left side (Mann Whitney, $p>0$ 1)

The interindividual median density of the nterior part of the septum, based in the case f each septum upon 12 anterior fields was 6 cells/field as compared with 54 cells/field n 12 posterior fields (Table III) This differ nce was not statistically significant ($p>0$ 1)

Anaesthetized material There was no sig nificant difference in the interindividual gob- et-cell density between normal and anaesthetized rabbits ($p=0$ 8) or between the right and left septum ($p>0$ 7) (Table III) or between the anterior and posterior segment of the septum ($p>0$ 1, Table III)

Operated material

1 *Occluded versus normal material* The interindividual density of all septa from the occluded side was significantly higher ($p<0$ 03) than that of all normal septa (right and left) The median density of the anterior segments of the septum was not significantly higher than of the posterior one ($p>0$ 05, Table III) The anterior and posterior segments of the septum respectively showed a significantly higher density ($p<0$ 025) than the corresponding segments in the normal material

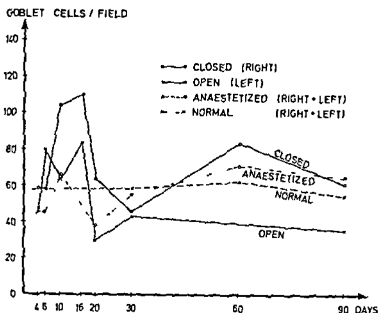


Fig 4 Interindividual median d... curves including all counting fields ... each septum in different materia...

2 *Open side versus normal material* The interindividual density of all septa on the open side was not significantly lower ($p>0.8$) than that of all septa (right and left) from the normal material (Table III) On the open side there were no differences in density between anterior and posterior segments of the sep... ($p>0.1$, Table III) The anterior and posterior segments of the septum respectively showed no significant difference in density as compared with the corresponding segments in the normal material ($p>0.05$)

3 *Occluded versus open side* The interindividual density of all septa on the occluded, right side was significantly higher ($p<0.01$) than of the open side The density in the 12 anterior fields on the occluded side was significantly higher ($p<0.01$) than in the corresponding anterior fields of the open side Similarly, the density in the 12 posterior fields on the occluded side was significantly higher ($p<0.025$) than in the corresponding fields on the open side

Density in Relation to Observation Period

Within the normal and anaesthetized materials no essential changes in density occurred during the observation period (Fig 4) Among the operated rabbits there occurred on the occluded side a distinct increase in density d... ing the first 16 days, followed by a ma... decrease until the 20th day, by which the d... sity had approached normal Then there ... a slight increase, so that after 90 days ... clusion of the nostril the density was only ... little higher than normal

Surprisingly, the open side also showed ... considerable increase in density up to the ... day and thereafter a decrease until the ... day After 90 days, the density was only a ... lower than normal (Fig 4)

Therefore, the changes in goblet-cell den... were assessed separately for the period ... days

The interindividual density during the ... riod 20–90 days after the operation was ... occluded side 62 cells as compared w... cells/field in the normal material This d... ence is not significant ($p>0.1$) On the ... side the density during this period was 42 ... field, which is significantly lower ($p<$... than in the normal material During the ... period, the density on the occluded side ... significantly higher than on the open side ($p<$ 0 004)

To ascertain whether the change in den... occurs predominantly in the anterior or ... posterior segment, the density during ... riod 20–90 days after the operation was ...

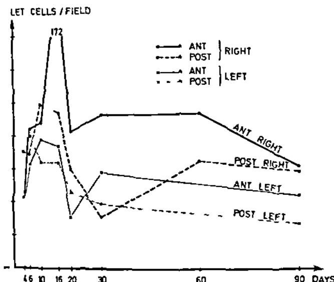


Fig 5 Interindividual median density curves representing the 12 anterior respectively the 12 posterior counting fields from each septum Right side is closed left side is open

lated separately On the occluded side there as anteriorly in the septum a significantly gher density ($p<0\ 05$) than in the same segent in the normal material On the other and, analysis of the corresponding posterior gments showed no significant differences in nsity ($p>0\ 05$) On the occluded side there as, during the period 20–90 days, a significantly higher density ($p<0\ 04$) anteriorly than osteriorly on the same side (Fig 5)

On the *open* side there was no significant fference in density anteriorly ($p>0\ 1$) as ompared with the normal material, but posa orly the density was significantly lower $p<0\ 025$) than at this site in the normal matnal Between the anterior and posterior segents of the open side there was no signifiant difference in density ($p>0\ 8$)

Anteriorly on the occluded side there was a ignificantly higher density ($p<0\ 025$) than an norly on the open side Posteriorly there as also a significantly higher density ($p<$ 025) on the occluded side than in the same ocation on the open side (Fig 5)

DISCUSSION AND CONCLUSION

According to Hilding (1932) there ought to be a considerable increase in goblet cell density on the occluded side and a considerable decrease on the open side Changes in density were said to be most marked in the animal occluded longest Our quantitative studies, however, did not show the pronounced changes reported in Hilding's publication, merely tendencies in the directions stated

(1) A few days after the operation a considerable increase in goblet cell density occurred on both sides of the nose, but it had worn off by approx the 20th day (Fig 4) This increase, which signifies increased differentiation of epithelial cells—presumably basal cells—can hardly be attributed to altered air flow, but rather to an irritative state caused by the operation Below, we shall therefore consider exclusively changes in density during the period 20–90 days, the *late period* during which only changes in air flow manifest themselves

(2) On the *occluded* side there was not, during this late period, any significant increase in density for the septum as a whole as compared with the *normal material* A certain tendency was observed, however, there being *anteriorly* on the septum a significantly higher density on the occluded side than in the same location in the normal material

(3) On the *open* side there was, during the

late period, a significantly lower density on the septum as a whole than in the *normal material* and this decrease was particularly marked in the posterior part of the septum

(4) The tendency towards higher *density on* the *occluded* and lower on the *open side* was more distinct, if these two sides *were com*pared During the late period *there was a sig*nificantly higher density *on the occluded side* both for the septum as a *whole and also when* comparing *only the anterior* and posterior parts *respectively*

(5) It must be concluded, therefore, *that a* completely abolished *air flow in the nose leads to a slight* increase and *doubling of the air flow* to a slight decrease in *goblet-cell density The changes in density manifest* themselves main*ly in two locations* (A) Anteriorly on the occluded side where previously *the effect of un*conditioned *respiratory* air was at a maximum *and where*, accordingly, *the occlusion* has *most effect* (B) *Posteriorly* on the open side, where a *doubled air flow* and poorer condition*ing of the air will be quite particularly* in evidence

In *human material we* have found parallels *Normal adults show anteriorly* on the septum *and conchae, where the* mucosa is affected by *unconditioned respiratory* air, the lowest gob*let cell density* (*Mogensen* & *Tos, 1977a, 1977b*) In laryngectomized patients who do not have nasal *respiration* a number of au*thors* (*Sternberg* 1924, Dixon *et al*, *1949*, Naumann, 1964 Puskas *et al*, *1970*) assessing sections of the nasal mucosa have found *more* numerous goblet cells than in normals *However*, there is not *agreement concerning* the degree of *goblet cell increase* again emphasizing the need for accurate *quantitative studies* of whole mount *specimens*

(6) A *rather interesting* finding is the in*crease in goblet cells* taking place 4 days after the operation (Fig 5) The *mechanical irrita*tion which is at a *maximum* on the right septum anteriorly, gives rise to an increase in density 2 or 3 times However *there is also* a question of a *systemic* action by the surgical procedure since goblet-cell de[illegible] *creased also posteriorly* on the ri[illegible] *and on the left septum*

A similar reaction may be expected *following* surgery on the nose or lar[illegible] *A rapid increase* in goblet-cell density [illegible] served in the Eustachian tube and m[illegible] under abnormal conditions (Tos & Bak*sen, 1977*) and must be assumed to occ[illegible] in catarrhal and infectious states of t[illegible] cosa

(7) *Determinations* of goblet-cell d[illegible] *carried out as blind studies* have show[illegible] *quantitative changes* in one of the m[illegible] components Other factors such as ch[illegible] in ciliary cells possible metaplastic chan[illegible] *the epithelium* changes in epithelial an[illegible] cosal thickness, and any processes in t[illegible] mina propria must also be studied qua[illegible]tively

After 3 months observation the goble[illegible] density on the occluded side was some[illegible] higher on the occluded and somewhat l[illegible] on the open side than in normal rabbits [illegible] 4) Therefore a longer observation per[illegible] needed to ascertain at which level the de[illegible] will become stabilized Moreover norma[illegible]tion of the air flow after re-opening the [illegible]cluded side will be able to elucidate whe[illegible]er, and if so when, normalization of the m[illegible]cosa takes place

ZUSAMMENFASSUNG

Bei Kaninchen wurde Vestibulum nasi auf einer Se[illegible] chirurgisch geschlossen Die Tiere wurden [illegible] Zeit höchstens 90 Tage beobachtet Schleimhaut [illegible] Nasenscheidewand wurde von beiden Seiten [illegible]men nach der PAS Alzianblau-Ganzpräparat [illegible] färbt und die Becherzellen gezählt Die D[illegible] Becherzellendichte zwischen der geschlossenen [illegible] offenen Seite war kleiner als es auf Grund der L[illegible] Angaben zu erwarten war Totale Ausschaltung des L[illegible]stromes während drei Monate führte nur zu [illegible] Vermehrung Verdopplung des Luftstromes [illegible] mäßigen Verminderung der Becherzellendichte [illegible] der ersten 16 Tage wurde eine bedeutende S[illegible] Dichte auf der geschlossenen aber auch auf der [illegible] Seite gefunden d e kaum als Folge der [illegible] mungsverhältnisse zu erklären ist

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BACTERIOLOGY OF MAXILLARY SINUSITIS IN RELATION TO QUALITY OF THE RETAINED SECRETION

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Abstract The bacteriological findings in 200 patients with acute maxillary sinusitis are reported It is concluded that the sampling technique—by antral aspiration—is highly significant in the evaluation of the bacteriological background of sinusitis whereas the anaerobic transport of the sample seems to be of less importance By aspiration the purulent secretion can be properly separated from the non purulent secretion Pneumococci *H influenzae* and anaerobic bacteria can be isolated in about 90% of the patients with true sinus empyema while sterile conditions are rare

During the years many studies have been devoted to the bacteriology of maxillary sinusitis Beta hemolytic streptococci, formerly a ... finding in sinusitis, are seen today ... sporadically while the occurrence of *Streptococcus pneumoniae* and *H influenzae* has increased These latter two species taken together are now isolated in 35 to 60% of the patients with acute maxillary sinusitis (Axelsson & Brorson, 1972, Nylen et al , 1972, Rantanen & Arvilommi, 1973, van Cawenberge et al , 1976) Concomitantly as the clinical interest in anaerobic bacteria has increased and the anaerobic culture technique has improved, anaerobic bacteria have been isolated in sinusitis in as high a frequency as 33% or more (Frederick & Braude, 1974, van Cawenberge et al , 1976) This high incidence of strictly anaerobic bacteria in purulent sinus secretions is plausible in the light of the extremely low oxygen tension in these secretions (Carenfelt & Lundberg, 1977) So far in all reports concerned with the bacteriological background of acute maxillary sinusitis it has not been possible to demonstrate bacteria in the sinus in 15 to 35% of the patients

With few exceptions the bacteriolog... analysis has been based on specimens ... from the lavage or from the nasal cav... has been claimed that these sampling ... niques are unsuitable as the risk of nasal ... tamination increases which is reflected ... high frequency of mixed flora Certa... irrigation also implies a dilution and ... genation of the specimens which can ... a low density of vital bacteria in the s... taken from the lavage When sinus ... was aspirated for bacteriological exam... the result was not related to the quality of ... secretion

The present paper reports the bacterio... logical findings in antral secretion obta... diluted by aspiration *from patients* ... *rather short history of sinusitis* ... tion has been paid to the relation between ... terial isolates and *the quality of the* ... sinus secretion as well as to the influence ... the transport technique used

MATERIAL AND METHODS

Antral secretions from 200 patients ... from maxillary sinusitis and not treated ... antibiotics during the previous mon... examined bacteriologically

In all cases the secretions were obtai... diluted by aspiration from patients ... through a Lichwitz needle inserted ... the inferior nasal meatus *The secret...* classified as purulent or non purulent ... ing to Carenfelt & Lundberg (1977)

le I *Bacterial isolates in 77 purulent and non purulent sinus secretions transported ob cally in sputum cups*

	Number of isolates	
	Purulent secre t ons	Non purulent secret ons
umococc	38	5
umococc B streptococci	1	
nfluen ae	11	1
ierob c bacteria	10	
erob c bacteria streptococci	1	
aerob c bacteria treptococci	2	
aerob c bacteria		
aph ep derm d s	2	
treptococc	5	
ph aureus	3	1
growth	4	6

During the first years of the investigation 968 to 1973 90 patients were selected for the udy as having retained sinus secretion in the axillary sinus both prior to and after 2–3 ays of antibiotic treatment The bacterio ogical examination was based on specimens spirated prior to the antibiotic treatment Only exceptionally had these patients a history of lingering sinusitis The remaining 110 pa ients studied during 1974 to 1977 were se ected as having retained secretion in the inus at least at the first examination None of hese patients had a history of sinusitis of long r than one month In patients with bilateral sinusitis the isolates of the clinically most seriously diseased sinus is reported Thus in all the sinus secretion of 200 sinuses from the same number of patients was bacteriologically analysed

Secretion samples collected during 1968 to 1973 were transported aerobically in sealed sputum cups without medium During 1974 to 1977 the secretion samples were transported in the following ways From 31 patients the as pirated secretions were transferred aerobically into agar tubes Samples of secretion from 28 patients were injected into anaerobic broth agar bottles From 51 patients samples of se cretion were transported both aerobically and anaerobically as described above The spec imens were usually managed at the laboratory within four hours and were never allowed to be stored for more than 12 hours

Bacterial isolation

During 1968 to 1973 the specimens were cul tivated on blood agar (Columbia agar base from BBL supplemented with 10% (v/v) horse blood) blood agar with 4 mg/l gentian violet endo agar (Oxoid) phenylethanol agar (Difco) supplemented with 5% (v/v) horse blood haematin agar (GC agar base sup plemented with IsoVitalex enrichment and 0 2% haemoglobin BBL) and a blood agar plate incubated anaerobically in a steel jar with a cold catalyst The blood agar with gentian violet was incubated anaerobically and the haematin agar in 5% CO_2 During 1974 to 1977 the specimens were cultivated as described above but with one exception For isolation of anaerobic bacteria a freshly prepared blood agar plate was incubated immediately in a Gas Pak jar (BBL) The anaerobic broth agar bot tles were incubated at 37°C and read daily un til either growth occurred or for 10 days after which time subculturing on anaerobic blood agar plates was performed

Haemophilus influenzae was identified on the basis of dependence on X and V factors Pneumococci and group A streptococci were identified by their sensitivity to optochin and bacitracin respectively and serotyped by cap sular reaction test and in the latter part of the study by immunoelectroosmophoresis (Wad strom et al 1974)

The DNase test was used to differentiate *Staphylococcus aureus* from *Staphylococcus epidermidis* Enteric bacteria were identified using the following biochemical reactions Production of acid and gas from mannitol hydrolysis of o nitrophenyl D galactopyrano side (ONPG) formation of indole urease and acetoin *Pseudomonas aeruginosa* was identi fied by the oxidase reaction and the ability to grow on agar containing 0 5 g/l Cetrimide

Table II *Bacterial isolates in 54 purulent and 28 non purulent sinus secretions transported aerobically in agar tubes*

	Number of isolates	
	Purulent secretions	Non purulent secretions
Pneumococci	16	7
Pneumococci *H influenzae*	1	
Pneumococci a streptococci	1	
H influenzae	16	2
Anaerobic bacteria	9	
Anaerobic bacteria a streptococci	3	
Anaerobic bacteria *Staph epidermidis*	1	
B streptococci	1	
a streptococci	2	
Branhamella catarrhalis	1	
Staph aureus	1	1
Staph epidermidis		1
Pseud aeruginosa Klebsiella		1
No growth	1	16

Table III *Bacterial isolates in 59 pur... 20 non purulent sinus secretions tra... in anaerobic culture bottles*

	Number of isol...	
	Purulent secretions	No... pur... secr...
Pneumococci	22	6
Pneumococci *H influenzae*	1	
Pneumococci a streptococci		1
Pneumococci *Staph epidermidis*		2
H influenzae	14	3
H influenzae Staph epidermidis	2	
H influenzae Pseud aeruginosa	1	
Anaerobic bacteria	11	
Anaerobic bacteria B streptococci	1	
Anaerobic bacteria *a streptococci*	3	
Anaerobic bacteria *Staph epidermidis*	2	
B streptococci	1	
a streptococci	2	
Branhamella catarrhalis	1	
Staph epidermidis		4
Pseud maltophilia		1
No growth		3

RESULTS

The bacteriological findings from 90 sinus secretions transported in sputum cups are ven in Table I In the purulent secretions predominating species was pneumococci, epresenting 49% of the isolates Anaerobic bacteria alone or together with other bacteria were present in 20% of the secretions *H influenzae* and Beta streptococci group A were isolated from 14 and 7% of the secretions, respectively In 5% of the purulent secretions no bacterial growth could be demonstrated Also in non purulent secretions the pneumococci were the most prevalent species found, in 5 of 13 cases In almost half of the non purulent secretions no bacterial growth could be demonstrated

A total of 82 samples of sinus secretion, 54 purulent and 28 non purulent were transported aerobically in agar tubes (Table II) In the purulent secretions pneumococci and *H influenzae* were almost equally common and taken together isolated in 34 of 54 secretions Anaerobes alone or taken together with other bacteria were isolated from 15 of 54 purulent secretions Only one purulent secretion w... out demonstrable bacteria was found Al... non purulent secretions were pneumoc... the most common finding In more tha... of these secretions (16/28) no bacterial g... could be demonstrated

Anaerobic broth agar bottles were ... for the transport of 79 samples of secre... 59 purulent and 20 non purulent secre... (Table III) Essentially the same bacterio... cal findings were encountered as wit... aerobic transport used In purulent secretio... pneumococci, *H influenzae* and anaer... bacteria made up the majority of isolates B... terial growth could not be demonstrated ... of the non purulent secretions

When samples of purulent secretion fro... sinuses were transported both aerobically a... anaerobically the results of the bacterial ... tions showed almost identical results f... two transport techniques (Table IV)

Table IV *Bacteriological findings in 37 purulent secretions aspirated from maxillary sinuses and transported aerobically and anaerobically*

	Transported	
	aerobically	anaerobically
Pneumococci*	12	12
H. influenzae*	13	14
Anaerobic bacteria	8	10
Miscellaneous bacteria	4	2
No growth	1	0

* In one secretion pneumococci and *H. influenzae* were isolated together

Considering all the 153 patients with maxillary sinus empyema from whom secretion samples were aspirated and transported aerobically, anaerobically or aerobically and anaerobically, pneumococci were present in 4?%, anaerobic bacteria in 23% and *H. influenzae* in 21% of the patients

DISCUSSION

The successful treatment of maxillary sinusitis necessitates a thorough knowledge of the prevailing bacteriology. In this study, special attention was paid to the relationship between the quality of the retained secretion, purulent or non purulent, and the bacterial findings. Furthermore, interest was focused on the extent to which the bacteriological findings are influenced by the transport techniques of the specimens from sampling time to their cultivation at the laboratory

All samples of sinus secretion in the present study were obtained by aspiration through a cannula introduced through the inferior nasal meatus By this technique the contamination of the specimens by bacteria in the nasal cavity and skin of the upper lip can be minimized as compared to specimens obtained by sinus irrigation This is amply demonstrated in the present study by the low frequency of mixed bacterial flora and staphylococci Another advantage of the aspiration procedure is the possibility of an accurate estimation of the severity of the sinusitis Thus, serous secretions and purulent secretions of low viscosity can hardly be distinguished when diluted in a large volume of saline A previous paper reported that the clinical classification of secretions as purulent and non-purulent corresponds to a clear-cut difference in bacterial and white corpuscular cell number (Carenfelt & Lundberg, 1978) In this paper the difference between purulent and non-purulent secretions is further emphasized by the fact that bacterial growth could be demonstrated in the purulent secretions from almost all the patients as opposed to the non purulent secretions Thus, in the latter secretions lack of bacterial growth was observed in 15 to 50% of the secretions, depending on the transport technique used This does not mean however, that non purulent secretions are sterile Six of 8 non purulent sinus secretions without demonstrable growth of bacteria, examined in a Bürker chamber, showed bacteria in numbers ranging between 1 1–7 3×10⁷/ml Corresponding figures for 16 purulent secretions were always >10⁹/ml Thus, no bacterial growth only means that the bacteria are unable to establish growth on the culture medium used

The method of transport of bacteriological specimens has long been a subject for discussion One possibility is to deliver the syringe with the aspirated secretion sealed by a rubber stopper, a method which preserves the anaerobic state of the purulent specimen and minimizes overgrowth of contaminating bacteria For practical reasons other methods had to be used in this study As opposed to samples taken with a cotton pin, a thin film of secretion and a high degree of aeration were avoided, which may explain the high frequency of anaerobic isolates The unexpected small differences in this study between aerobically and anaerobically transported samples as regards to the distribution of bacteria support this opinion However, overgrowth of facultative bacteria in the anaerobic broth agar

bottles may have concealed an even higher incidence of anaerobic bacteria

The frequent occurrence of pneumococci and *H influenzae* in acute maxillary sinusitis found in this study is in accordance with most previous reports It was recently reported that *H influenzae* has become a more common finding in sinusitis during recent years (van Cawenberge et al , 1976) In the present study *H influenzae* was isolated from purulent secretions in a higher frequency during the second part of the study (30–35%) as compared with the first part (14%) This discrepancy, however, does not necessarily reflect a true frequency increase of this species The relative increase of *H influenzae* may merely reflect a higher degree of curability in sinusitis caused by this bacteria, as in the early part of this study only patients with retained antral secretion 2 to 3 days after sample taking were selected

In contrast to earlier studies, van Cawenberge et al (1976) found anaerobic bacteria to be a common cause of acute sinusitis In the [illegible]esent material anaerobic bacteria as a group, [illegible]her alone or with various other bacteria, were the bacteria most frequently occurring in purulent secretions, next to pneumococci, which clearly indicates the importance of an aerobic bacteria as pathogens also in acute purulent sinusitis This conclusion is further supported by the fact that anaerobic bacteria were never isolated together with pneumococci or *H influenzae* both well known pathogens

As demonstrated in the present report, bacteria can almost invariably be isolated from purulent antral secretions when aspirated for examination In contrast the isolation of bacteria from non purulent secretions is often unsuccessful, but this also depends on the method of transport of the sample Taken together, pneumococci, *H influenzae* and anaerobic bacteria can be isolated from the sinus in about 90% of the patients with acute ma[illegible] sitis This implies that comparative[illegible] terial species have to be suspected [illegible] lecting antibiotics for the treatment

ZUSAMMENFASSUNG

Der bakteriologische Befund bei 200 Pati[illegible] maxillarer Sinusitis wird hier aufgezeig[illegible] Schluß gezogen daß die Techn[illegible] der Pr[illegible] – durch antrale Aspiration – für die [illegible] bakteriologischen Grundlage von S[illegible] gnifikant ist während der anaerobische Tr[illegible] Probe von geringerer Bedeutung zu sein [illegible] Aspiration läßt sich das eitrige Sekret von [illegible] eitrigen Sekret zweckmäßig trennen Pneu[illegible] *influenzae* und anaerobische Bakterien k[illegible] 90 v H der Patienten mit echtem S[illegible] werden hingegen sind sterile Verhältnisse [illegible] treffen

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ASPERGILLOSIS OF THE MAXILLARY SINUS

Clinical and Histopathological Features of 4 Cases and a Review of the Literature

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ract Four patients with aspergillus maxillary sinuitis eported and a review of the literature on aspergillus nasal sinu tis during the last 10 years is presented patients had symptoms and X ray findings similar to on c bacterial sinuitis One patient had been operated 6 years previously due to a chronic sinuitis and 3 e patients had been treated with broad spectrum anti drugs Caldwell Luc operations were performed to exclude malignant tumours The aspergillus etio- of the sinuitis was discovered in routine stained sec by histo-pathological examination of the sinus mu e No recurrence of the fungus infection occurred the Caldwell Luc operation which was valuable for correct diagnosis as well as being the best suited py

igus infection caused by aspergillus is a disorder in man in industrialized coun- s The most important site of infection is lung with or without hematogenous spread ther organs (Emmons et al , 1963, Young l 1970) A localized aspergillus infection also occur in the upper respiratory tract, is probably more common than was previ ly suspected (Iwamoto et al , 1972 Gri iu et al , 1977) To our knowledge only 3 ients with sinuitis caused by aspergillus e been reported from Scandinavia (Rohrt, 4 Andersen & Stenderup 1956, Mikael 1975) Consequently, and because of the icurrent diagnostic difficulties, we want to ort 4 additional cases

iew of the literature

cattle, sheep and birds, aspergillus infec ns are common For example, in horses aspergillus is the etiologic agent in a sinuitis like condition, guttural pouch mycosis where the clinical picture is dominated by purulent discharge from the nose and invasion of blood vessels, often leading to fatal epistaxis (Cook, 1968, Bjorklund & Pålsson, 1970, Lingard et al , 1974)

In man, aspergillus infection of the maxillary sinus can behave either as an invasive or non-invasive infection (Hora, 1965) In the non invasive infection the symptoms, clinical signs and X ray findings are similar to a chronic bac terial sinuitis In the invasive form, bone destruction and involvement of the orbit often render the clinical differentiation from a malig nant tumour difficult (Chapnic & Bach, 1976)

Infections due to aspergillus in the nose and paranasal sinus, since the review of Sandison et al (1967), are summarized in Table I

The cases reported by Sandison et al (1967) were included and reinvestigated in the articles by Milošev et al (1969) and Veress et al (1973)

Most authors report solitary cases of aspergillus infection of the paranasal sinuses, but in a recent systemic histopathological and mycological investigation, aspergillus sinuitis was seen more frequently (Grigoriu et al , 1977) In the Sudan the disease appears mostly as a granuloma with giant cell reaction, fibrosis and often involvement of the orbit (Sandison et al , 1967, Milošev et al , 1969, Veress et al , 1973) The hot climate with a low humidity, toxic

Table I *Review of reported aspergillus paranasal sinusitis during the last 10 years*

Authors	No of pats	Age and sex	Symptoms	Site	Treatment
Milošev et al (1969) Sudan	17	11 males 6 females	Proptosis Painless swelling of the ethmoid and/or maxillary region	Paranasal sinus and orbit	[illegible]
Taillens et al (1970) Switzerland	1	F 35	Tubal obstruction with otosalpingitis Chronic maxillary sinusitis	Maxillary sinus	[illegible]
Zinneman (1972) USA	1	M 46	Unilateral pain rapidly declining vision Mucosanguineous nasal discharge Proptosis	Maxillary sinus and orbit	[illegible]
Iwamoto et al (1972) Japan	1	M 60	Nasal obstruction Fever Purulent rhinorrhea Cheek pain	Maxillary sinus	[illegible]
Grigoriu & Dutoit (1973) Switzerland	1	M 53	*Maxillary sinusitis*	Maxillary *and ethmoid* sinuses	[illegible]
Veress et al (1973) Sudan	46	25 males 21 females	Swelling of the maxillary and/or ethmoid sinuses Proptosis	Paranasal sinus and orbit	[illegible]
Mikaelsen (1975) Norway	1	M 41	Cheek pain Unilat rhinorrhea Sneezing	Maxillary and ethmoid sinuses	Caldwell-Luc
…ch et al (1975) France	2	F 74	Unilateral nasal obstruction Purulent and hemorrhagic rhinorrhea Headache	Paranasal sinus	[illegible]
		F 22	Unilateral purulent rhinorrhea Cacosomia	Maxillary sinus	[illegible]
Warder et al (1975) USA	3	F 67	Facial pain	Maxillary sinus	[illegible]
		F 63	Nasal obstruction Hemorrhagic rhinorrhea		
		M 18	Proptosis	Fronto-ethmoid complex	[illegible]
Gonty & Page (1977) USA	1	M 26	Cheek pain	Maxillary sinus bilat	[illegible]
Grigoriu et al (1977) Switzerland	43	18 females 25 males	Nasal obstruction Rhinorrhea Orbital pain Headache	Maxillary sinus	[illegible]

imen logy	Mycology	Remarks
ranuloma tate hyphae cells	*A flavus* cultured	
-green anch ng	*A niger* cultured	
ous tissue inflam muco- hyphae inter s Mucor	*A flavus* and *Nocardia asteroides* cultured	Cutaneous fistulae in the canine fossa
wn mass tes assumed to rg llus	–	
mass m suggested lus	*A fumigatus* cultured	
c grey te mass tubercles with necrosis hyphae and in tory cells with ls	*A flavus* cultured	Prolifera tive form Exudative necrotiz ing form Mixed form
ee lium o Asperg llus	–	
f id *llus mycelia*	–	Immuno el phoresis *A nidulans*
brown foreign body ma *Aspergil a*	*A flavus* cultured	Tooth ex traction 6 months previously
thickening lent necrotic lloma	–	
fouli us ball of hyphae	*A fumigatus* cultured	Op max frac ture 6 years previously Caldwell Luc 2 years pre viously due to chron max
nflamed mu th mycelia	*A fumigatus* (10) *A niger* (2) *A flavus* (1) cultured	Mucopuro- lent form Budding and/or ca seous form Pseudotu mor form

metabolites of the fungus and the local immunological conditions are discussed as a possible explanation for this concentration of the disease in the Sudan (Sandison et al, 1967, Veress et al, 1973) For us, another possible explanation could be the living habits and the environmental conditions in parts of the Sudan, where among the Nuer, for instance, the carcasses and bodily products of the cattle are widely used for household purposes Cattle dung is used for plastering walls and floors of straw huts in cattle compounds, as plaster to protect wounds and as tooth powder (Evans-Pritchard, 1940) From the report by Milošev et al (1969) it is also evident that many of the patients were working as farmers and aspergillus species were isolated from the environment of the patients (Sandison et al, 1967)

CASE REPORTS

The clinical features of the 4 cases all with left sided involvement of the maxillary sinus studied in the present investigation are summarized in Table II

There was a considerable age variation in the material consisting of 1 female and 3 male patients The female had a previous history of Caldwell Luc operation 6 years earlier due to a chronic maxillary sinuitis after a tooth extraction with sinus perforation The 3 male patients had been in good health None of the patients suffered from a malignant or systemic disease The duration of symptoms before the present operation varied markedly between the patients One patient (M 80) had symptoms of bad smell from the nose for 3 years The first symptom of the disease in one patient (M 61) was an acute attack of epistaxis Sinus X ray revealed mucosal swelling or opacification All 3 male patients had been treated with tetracycline for their sinuitis Due to persistent symptoms and in spite of a regime of nasal decongestant antibiotics and lavage all 4 patients were operated *ad modum* Caldwell Luc The mucosal lining of the sinuses were found to be edematous and markedly thickened In 2 of the patients greyish necrotic masses were observed and in one of these (M 80) the medial sinus wall was replaced by necrotic tissue

Histo-pathology

Microscopic examination of the material removed at Caldwell Luc operation revealed an inflamed respiratory mucosa diffusely invaded by numerous polymorphonuclear granulocytes and mononuclear inflammatory cells Eosinophilic granulocytes were not very conspicuous Separated from or situated on the surface of the pseudostratified ciliated columnar epithelium separate and









Fig. 1a Micrograph of inflamed maxillary sinus mucosa with adjacent, partly necrotic fungal mycelia Hematoxylin-eosin, ×40

Fig 1b Maxillary mucosa with suspicion of fungal invasion Hematoxylin-eosin, ×100

Fig 1c. Fungal mycelia showing dichotomous branching [illegible]

dichotomous branching hyphae with conidiophores were observed (Fig 1*a*). Occasionally the mycelia were suspected of infiltrating the mucosa, as depicted in Fig 1*b* No granuloma or giant cell reaction with fibrosis could be detected The pigmented conidiophores ended in a gradually widening vesicle (Fig 1*c*) The upper part of the vesicle (capitulum) was covered with a single layer of parallel arranged primary sterigmata. Spherical conidia, sometimes forming unbranched chains, were observed in the vicinity of the fruiting bodies (Fig 1*d*) (Thom & Raper, 1945) No cultures were performed but the mor- [illegible]

et al , 1963) The diagnosis of the mycotic infection could be made in sections stained with hematoxylin-eosin or according to van Gieson Staining with [illegible] and PAS are not necessary for the [illegible] diagnosis

DISCUSSION

Aspergillus infection of the maxillary [illegible] be found in patients in general good [illegible] nutritionally deprived persons and in [illegible] with an underlying debilitating disease [illegible] mons et al., 1963). Systemic diseases, such [illegible] collagenosis and malignant tumours, and [illegible] treatment with immunosuppressive drugs [illegible] cytostatic agents, have been associated [illegible]

ble II *Cases of aspergillosis of the maxillary sinus*

ent	Predisposing factors	Symptoms	X ray	Treatment	Follow up
ale	Chronic max sinuitis after tooth extraction and Caldwell Luc op 6 years previously	Cheek pain na sal discharge Rhinorrhea	Opacifi cation	Caldwell Luc	Control after 3 months and telephone contact after 3 years no symptoms
e l	Tetracycline treatment	Cheek pain Epistaxis	Opacifi cation	Caldwell Luc	Control after 9 months and telephone contact after 5 5 years no symptoms
e	Tetracycline treatment	Unilateral nasal dis charge Cacosomia	Mucosal thicken ning Fluid	Caldwell Luc	Control after 1 month no symptoms
e	Tetracycline treatment	Cacosomia	Opacifi cation	Caldwell Luc	Control after 3 months no symptoms

increased incidence of fungal infections oung et al , 1970) In patients in general od health there are probably some factors, stemic or local, predisposing to the fungal ection of the paranasal sinus (Chapnic & ch 1976) Most patients reported have been ated with broad spectrum antibiotics, which ppress the bacterial flora and support a fun- l overgrowth Recurrent bacterial infec- ns tooth extraction trauma or surgery may ect the drainage of the sinus

Our 4 patients were all in good health, ex- nt for their E N T symptoms Three of the tients had been treated with lavage and sys- mic tetracycline drugs without improvement the disease prior to operation One patient 36) had 6 years previously been operated *ad odum* Caldwell Luc for a sinus infection ter a tooth extraction In all patients the pergillus infections were of the non invasive pe except for the oldest patient (M 80) here the medial wall of the maxillary sinus as destroyed and at operation found to be placed by a "necrotic granular mass" Histo thological examination of the tissue re- oved from this patient revealed a suspicious ngus infiltration in the mucosa However, is elderly patient did not suffer from any de litating disease or nutritional deficit, but the ymic involution and cellular immune dys- nction known to occur in elderly persons could have caused decreased host defence and enhanced a destructive infection

In none of the patients was the mycotic in fection suspected preoperatively, and no cul tures with respect to fungus were performed The etiology of the sinuitis was obvious first upon histo-pathological examination of the surgically removed material Due to the my- celial structure and the presence of fruiting bodies the diagnosis of aspergillus infection could be made on routine stained sections The Caldwell Luc operations have been of diagnostic importance as well as the therapy of choice, as not all infections of the paranasal sinuses can be satisfactorily treated by lavage and antibiotics No other treatments were given after the operations and all patients were free from symptoms at follow-up, indicating that with Caldwell Luc operation no addi tional local or systemic drug therapy is neces sary

The clinical conclusions from the review and our reported cases are (1) A maxillary sinuitis which is not improved by lavage and oral antibiotic therapy should be subjected to diagnostic Caldwell Luc operation (2) The diagnosis of aspergillus infection can be es- tablished by histo-pathological examination of routinely stained sections (3) The Caldwell Luc operation is also the therapy of choice in cases of aspergillus maxillary sinus infection

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ZUSAMMENFASSUNG

Vier Patienten mit Aspergillusinfektion der maxillaren Nebenhohle und eine Literaturubersicht von Aspergillusinfektionen der Nasennebenhohlen der letzten 10 Jahre werden präsentiert Symptome und Röntgenbefunde der Patienten sind ähnlich einer chronisch bakteriellen Sinuitis Ein Patient wurde vor 6 Jahren wegen einer chronischen Sinuitis operiert und drei Patienten hatten Behandlung mit Breitspektrumantibiotika bekommen Operation ad modum Caldwell Luc, auch um einen malignen Tumor auszuschließen, wurde bei allen Patienten durchgefuhrt Die Aspergillusätiologie der Sinuitis war bei der *mikroskopischen Routineuntersuchung der* Sinusmucosae erfaßt Kein Rezidiv der Pilzinfektion konnte nach der Operation festgestellt werden Die Caldwell Luc Operation brachte die korrekte Diagnose und war gleichzeitig die adäquate Therapie

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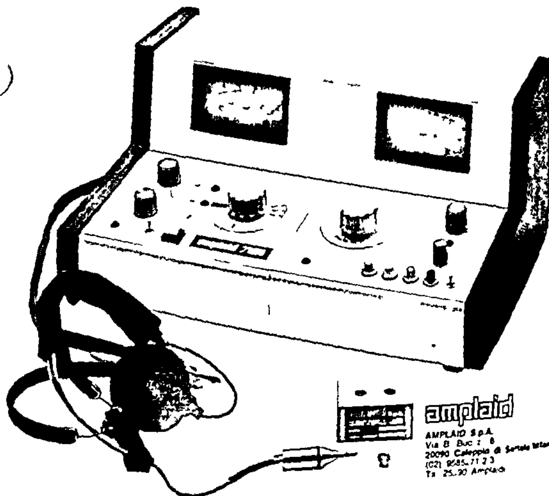
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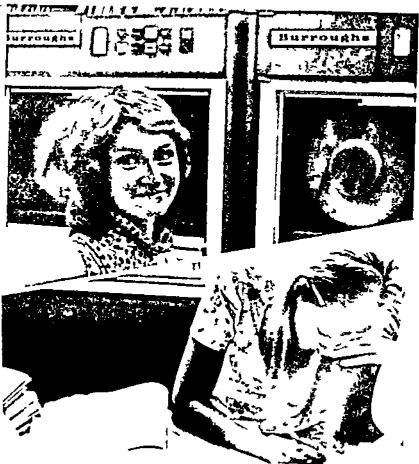
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compliance and acoustic reflex meter

Compliance
Contralateral reflex
Ipsilateral reflex

- d rect read ng compl ance meter n cc
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- for con ra- and ps lateral re ex e ng pure tone s mu (0 5 1 2 and 4 K H) a d b oad band no se as wel as low and h gh-pass f tered no se
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- output to X Y p otter and str p-chart recorder





Burroughs
Burroughs

Acta Otolaryngol 86 309-313, 1978

THE EFFERENT INNERVATION IN THE REGION OF INNER HAIR CELLS IN THE ORGAN OF CORTI

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tract The efferent innervation of the inner hair cells orti s organ of the laboratory rat and of a bat was stigated with help of histochemical staining of the r spiral bundle The area of acetylcholinesterase tive fibres in the inner spiral bundle regions was meas- d. The bulk of the efferent fibres lie in the middle part he cochlea Towards the apical and lower basal turns sinct decrease in the number of fibres was observed

contrast to the data available on the efferent ervation in the region of outer hair cells re is little data available on it in the inner r cells By means of a histochemical method staining an attempt was made to obtain a neral view of efferent fibres in the inner hair l region This study was suggested by Pro- sor Klinke, Frankfurt Competent struc- es of efferent innervation in this area are, st and foremost, the region of the inner ral bundle (inner spiral plexus), the spiral nel bundle, and direct efferent endings at inner hair cells Efferent innervation is ctionally effective either in the receptors or the afferent fibres originating there This dy is especially concerned with the inner ral bundle

MATERIAL AND METHOD

e efferent fibres in this region were marked th acetylcholinesterase (AchE) The prepa- tions of AchF were made by incubating the viously fixed and frozen sections of rats d two species of bats (Microchiroptera) ac- ng to Karnovsky s method The incuba periods were generally long (up to 24 hours) In this way the positively reacting fibres were made quite distinct The prepara tions were evaluated particularly with regard to the arrangement of the efferent fibres in the individual parts of the cochlea The diameters of the precipitate planes were measured, and the areas calculated from these diameters us ing the circle or ellipse formula (Fig 1) The area was taken to be an indication of the in nervation density

RESULTS

Before considering the results, a brief discus sion of the structure and composition of the inner spiral bundle (ISB) is necessary According to investigations made by Smith (1967), Smith & Takasaka (1971), Spoendlin (1973, 1975) and others, the inner spiral bundle consists of a network of predominantly efferent fibres which are closely connected with af ferent dendrites These afferent dendrites originate mainly from the inner hair cells Besides the efferent fibres, some afferent spiral neurons run within the ISB (Spoendlin, 1969, Sobkowicz et al , 1975, Perkins & Morest, 1975) According to the investigation of Wright & Preston (1975) the ISB and the spiral tunnel bundle have a close structural relationship In our study we were not able to evaluate the spiral tunnel bundle

The place of origin of efferent fibres and how they form synapses with other structures seems to be of special significance for the explanation of our own findings The origin of

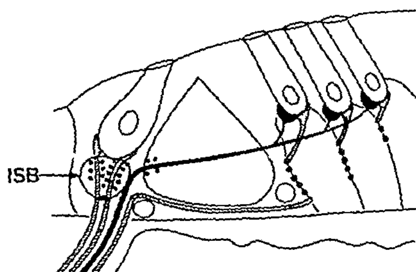


Fig 1 Innervation of Corti's organ (after *Spoendlin* 1973) The inner spiral bundle (*ISB*) is surrounded with a circular line

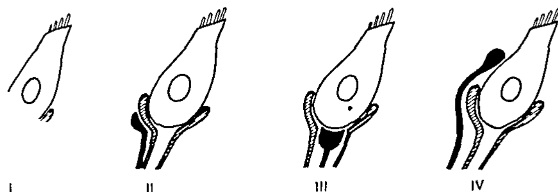
the efferent fibres in the ISB was investigated by experimental severing of those fibres in various mammals Table I shows some of these findings *From them we can see that*, depending to a certain degree on species and part of the cochlea, a greater or lesser number of ISB fibres originate from the uncrossed olivocochlear bundle (UOCB) When transecting the crossed olivocochlear bundle (COCB) it appears that the *main part of the efferent* fibres in the ISB (inner spiral bundle) of the rat stems from the uncrossed OCB as already described by Iurato (1964) From our own experiments, midline brain stem lesions in rats showed that the COCB runs to the outer hair cells and to the basal part of the ISB The apical ISB on the other hand comes mainly from the UOCB This is also in agreement with the results of Warr (1975), who found that 60% of the efferent fibres in the kitten originated from the ipsilateral side Ross et al (1977), however, have doubts about the Warr's method the uptake of horseradish peroxidase does not seem to be specific for the efferent olivocochlear neurons in the brainstem

The second point of importance for evaluation is the formation of synaptic structures which have been identified in various locations in connection with the efferent fibres of the ISB In our own preparations for light microscopy, the existence of axosomatic structures could of course not be directly verified from the precipitates A comparison of the preparations suggests that with rats the precipitates are nearer to the inner hair cells than with the bats

Species dependency in connection with axodendritic synapses has already been described with regard to electron microscopic investigations Angelborg & Engstrom (1973) and Spoendlin (1966) point to the presence of axosomatic synapses in rodents, whereas practically only axodendritic synapses could be identified in other mammals Figure 2 shows diagrams of the various findings Diagrams I and II illustrate the varying positions of the axodendritic synapses, diagrams III and IV show those of the axosomatic synapses I is to be postulated according to Spoendlin with the cat (1973), II according to Smith (1967) III according to Angelborg & Engstrom with the

Table I *Origin of efferent fibres in the inner spiral bundle*

Species	COCB	UOCB
Squirrel monkey (Nakai & Igarashi 1974)	70% basal	–
Cat (Spoendlin 1973)	50% basal	50%
Chinchilla (Iurato et al 1968)	–	100%
Guinea pig (Wright & Preston 1973)	+	+



2 Efferent synaptic structures (black) on inner hair
s

nea pig (1973), and IV according to Wright
Preston (1973) and according to the SEM
ations of Bredberg in the rabbit (1976)
A third point, which has to be considered
the explanation of our histochemical find
concerns the sediment areas of the reac
products Ultrahistochemically, acetyl-
linesterase activity is identified in the axo
lasma membranes of the efferent fibres and in
eir presynaptic expansions (Iurato et al ,
'71) The light microscope can only give a
ugh view of the distribution of the ferment
he area of the sediment, however, could be
ermined relatively precisely, and measure
ents yielded identical results for the individ
section series In accordance with the
ork of Spoendlin (1970), who found widely
arying quantities of internal spiral fibres in
basal turns of cats, our own tests produced
idely varying diameters of the precipitate
in the basal turn Table II gives the re
ts of the precipitate planes in the region of
inner spiral bundle in the individual turns
he figures for the areas (in μm^2) were ob-
ained by an average value calculation for the
idual turns of 5 rats and three specimens
f *Barbastella barbastellus* We found a low
of ferment sedimentation in the ISB in the
asal and apical turns The lowest value was
easured in the basal turn (hook part) at 6
, the smallest area in the apical part
ounted to 14 μm^2 Because of their size, the
s yielded lower values In the case of the
bat a more pronounced decrease, both apically and basally, could be noted in the efferent fibres of the outer hair cells, than was the case in the efferent fibres in the ISB, as far as the histochemical preparations could demon strate Perkins, in 1973, described a stronger efferent control in the region of the inner hair cells of cats and rats by using Golgi preparations

DISCUSSION

The present study attempts to evaluate the efferent fibres of the ISB in the individual cochlear turns, by using histochemical meth ods Hitherto the only available findings were those of Hilding & Wersall (1962) and of Ishii & Balogh (1968) Hilding & Wersall described an apically weaker reaction in the ISB of gui nea pigs, whereas Ishii & Balogh speak of the same reaction strength in all turns As pos tulated by Klinke & Galley (1974) efferent in nervation in the region of the inner hair cells

Table II *Section areas (mean values in μm^2) of the inner spiral bundle in the different parts of the cochlea*

Turn	Rat	Bat
Lower basal	61	11
Upper basal	196	35
Middle	163	42
Apical	72	28

does not seem to be the same in all turns The basal and apical decrease corresponds to the decrease in the efferent innervation in the outer hair cells, although not in every respect (Ishii & Balogh, 1968, Spoendlin, 1969) Fex (1973) postulated control mechanisms, which influence the outer and inner hair cells in the same way in order to explain the findings of Wiederhold & Kiang (1970) In this connection, however, it is necessary to explain how much influence crossed and uncrossed efferent fibres have in the formation of the ISB in the individual species

The present study in the rat led to the same result as was obtained by Wright (1975) in the guinea pig with a silver impregnation technique The greater part of the ISB lies in the middle cochlea The bundle is markedly reduced in size in the apical and lower basal turns Fex & Wenthold (1976) measured the choline acetyltransferase activity in the guinea pig cochlea The activity was higher in the [illegible] turn than in the fourth turn and was [illegible]er, when the region of the inner hair cells [illegible] included in the preparations These findings support the hypothesis that the olivocochlear fibres are cholinergic and also that in the middle part of the cochlea the inner hair cells have a stronger efferent innervation

ZUSAMMENFASSUNG

Die efferente Innervation der inneren Haarzellen im Cortischen Organ wurde mit Hilfe der Darstellung Acetylcholinesterase positiver Fasern im inneren Spiralbundel der Ratte und einer Fledermausart untersucht Es ergab sich gemessen an der Fläche der Fermentablagerungen im mittleren Teil der Cochlea die starkste efferente Innervation der inneren Haarzellen Gegen die Spitze und besonders gegen die untere Basalwindung nimmt die Zahl der efferenten Fasern im inneren Spiralbundel deutlich ab Der Ursprung der Fasern im inneren Spiralbundel der Ratte liegt fur den apikalen Teil der Cochlea vorwiegend im ungekreuzten olivo cochlearen Bündel Die Wege der efferenten Innervation im Gebiet der inneren Haarzellen (direkte Synapsen an den Haarzellen der Synapsen an den afferenten Dendriten) werden diskutiert Die Ergebnisse der vorliegenden Untersuchung stehen mit den Resultaten anderer histologischer und biochemischer Untersuchungen in Einklang

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GLYCOGEN ACCUMULATION IN REISSNER'S MEMBRANE FOLLOWING CHEMICAL SYMPATHECTOMY WITH 6-HYDROXYDOPAMINE

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Abstract Rats were chemically sympathectomized by injecting them with 6-hydroxydopamine repeatedly during early postnatal development Litter mates left untreated or injected with the vehicle only were used as controls Inner ear tissues from a series of treated and control rats 11–72 days of age were fixed then microdissected and prepared by usual methods for transmission electron microscopical study Control rats 11 days of age showed accumulation of glycogen particles in Reissner s membrane particularly in the epithelial cell layer near its attachment to the spiral limbus The epithelial cells in that region also had rough endoplasmic reticulum with greatly widened cisternae containing a granular substance The glycogen was not present after 11 days and the cisternae diminished in size with further postnatal development The epithelial cells of the membrane in controls of all ages had intercellular clefts that were closed at the endolymphatic surface by zonulae occludentes open narrowly at their basal end and variously dilated in between Coated vesicles were common at apical and lateral cell borders as well as intracellularly Treated rats on the other hand showed an accumulation of glycogen particles in the epithelial cells that increased between 11 and 32 days and persisted in some cells at 72 days The glycogen particles β in type were electron dense ~150–300 Å in diameter and often aggregated in large masses particularly in the lateral cell regions Concomitantly with glycogen accumulation coated vesicles diminished numerically and the intercellular clefts narrowed becoming of uniformly narrow dimensions by 32 days An upturn in the number of coated vesicles and a widening of the intercellular clefts occurred by 72 days The results are interpreted as indicating that the epithelial cells of Reissner s membrane have an energy dependent coupled ion/water transport capability that is ordinarily supported by glucose metabolism It appears that the glucose metabolism is in turn regulated by catecholamines suggesting the existence of β adrenergic receptors on the epithelial cells of Reissner s membrane

Experimental manipulation of the adrenergic nervous system of the rat by use of 6 hydroxydopamine, a substance known to induce chemical sympathectomy under appropriat conditions (Tranzer & Thoenen 1967 1968 Thoenen & Tranzer, 1968 Malmfors & Sach 1968, and others), was carried out in order t learn whether or not sites of catecholamin influence on inner ear function would be r vealed Because the inner ear is normal endowed with a rich supply of adrenerg nerve fibers, as first documented by Spoen lin & Lichtensteiger (1966), it was expect that the absence of circulating catecholamin might cause ultrastructural changes in speci target cells, or signs of abnormal developme or of deterioration of the organ of Corti seemed highly unlikely that so abundant a adrenergic innervation could be without in portance for the inner ear, although convin ing experimental evidence of the function significance of its sympathetic nerve supply still lacking

It is well recognized that catecholamines a involved in the regulation of glycogen metab lism in other regions of the body Thus fa only two cochlear tissues have been identifi as storage sites of glycogen, the stria vasc laris and the organ of Corti, on the basis histochemical (Belanger, 1957, Vinnikov Titova, 1957, 1964, Falbe Hansen 1963 19 Falbe-Hansen & Thomsen, 1963) and micr chemical (Matschinsky & Thalmann 19 Thalmann, 1971, 1975) determinations B tissues also store glucose (Matschinsky Thalmann, 1970, Thalmann 1971, Thalma et al , 1972)

Only rarely has glycogen been described in cochlear cells at the ultrastructural level This may be due in part to the difficulty often encountered in preserving and staining glycogen unless certain procedures are followed (Revel et al, 1960, de Bruijn, 1973) Hilding et al (1977), utilizing a method recommended by de Bruijn documented the onset and course of glycogen storage in the developing rat cochlea They found that glycogen appeared in abundance first in Reissner's membrane in the fetus (day 17), then in the pillar cells and the stria vascularis, disappearing from these areas by 10 days after birth In the adult rat, glycogen occurred in a finely dispersed state in the outer hair cells with no detectable gradient from base to apex other than could be accounted for on the basis of cell size

No previous histochemical, microchemical or ultrastructural findings have suggested that glycogen metabolism or storage occur in Reissner's membrane except as a relatively brief developmental phenomenon This report deals with ultrastructural evidence of extensive glycogen accumulation by the epithelial cells of Reissner's membrane after chemical sympathectomy with 6-hydroxydopamine, with concomitant changes in the numbers of vesicles and the size of the intercellular spaces

MATERIAL AND METHODS

Thirty two Sprague-Dawley rats were injected with 6-hydroxydopamine (50 μg/kg body weight) according to one of the two following schedules One group of 21 rats was injected on the day of birth, then every other day until a total of 4 doses was given (last dose on day 7) Another group of 11 rats was injected on day one, then every other day for seven doses, followed by an injection every fifth day until day 30 or sacrifice, whichever came first Eleven litter mates were used as controls, 8 of these rats were left untreated and 3 were injected with the vehicle only (ascorbic acid in physiological saline) The purpose of the multiple injections was two-fold to induce as complete a sympathectomy as possible and to guard against the possibility of regeneration of functional adrenergic terminals for a significant period of time

A series of inner ears from treated and control rats was obtained by sacrificing animals on days 11, 17, 22, 28, 32, and 72 after birth Animals were first anesthetized with chloral hydrate, then perfused systemically by the intracardiac route with a mixture of 3% glutaraldehyde, 4% paraformaldehyde in Millonig buffer (pH 7 35) Next, the cochleas were dissected in additional fixative and opened to allow better penetration of fluids The tissue was then decalcified in EDTA glutaraldehyde (Baird et al, 1967) and post fixed in 1% osmium tetroxide in Millonig buffer The tissues were dehydrated and embedded in Epon according to routine procedures

Eight treated rats covering the time periods noted above were chosen at random and used in this report Five litter mates were studied as controls The embedded tissues from these rats were thick-sectioned and stained with toluidine blue for orientation purposes The blocks were trimmed, thin sectioned, and the sections were mounted on grids, stained with uranyl acetate and lead citrate (Reynolds, 1963), and studied in a Siemens Elmiskop IA transmission electron microscope

Overt signs of chemical sympathectomy

All treated animals were observed for physiological and/or behavioral changes that might be indicative of chemical sympathectomy Treated rats demonstrated certain signs of autonomic imbalance that varied in severity from one animal to another The most frequently observed signs included excessive salivation, frequent defecation, and dribbling of urine After mild stimulation, the treated young animals often fell asleep while control animals returned to grooming and exploratory behavior The most surprising effect was that

ated animals were often difficult to anes-
-tize requiring two or more times the
ount of anesthetic necessary for controls

RESULTS

Control Animals

e general ultrastructure of Reissner's mem-
ane is well known and need not be described
re Certain details are presented, however,
order to consider fully the changes which
curred in the membranes of animals treated
th 6-hydroxydopamine

esothelial cells

control animals, the mesothelial cells of
issner's membrane were thin and sheet-like
cept for the region of the nucleus which
lged into the perilymphatic space (Fig 1)

1 The typical two-layered structure of Reissner's rane is shown in this electron micrograph The epi lial cells (*EC*) are attached to one another apically by a nctional complex (*JC*) often consisting of a zonula oc dens a zonula adherens and a macula adherens (des osome) A short distance below this complex, the inter llar space

(*ICS*) are common at all cell border membranes The ihelial cells are bounded basally by a basal lamina L) Between this lamina and the undulating basal bor rs of the mesothelial cells (*MC*) is a space of varying mensions containing a small amount of delicate, fila ntous material (*S*) The mesothelial cells often overlap e another (*arrowheads*) but junctional complexes are king The mesothelial cells contain the usual types of ganelles although they are scantier than in the epithelial lls Numerous uncoated vesicles (*UV*) are seen at all undary membranes The bar indicates 1 µm on this and following electron micrographs 28-day-old normal rat

Fig 2 The region of the junction of Reissner's mem ane with the limbus is modified in young animals The ithelial cells (*EC*) of Reissner's membrane contain ugh endoplasmic reticulum with greatly widened cis rnae filled with a granular material (*arrows*) This same pe of rough endoplasmic reticulum is found in the first w epithelial cells continuing over the spiral limbus L) Glycogen patches (*GLY*) are common in the epi elial cells of the junction region Some of the epithelial ells of the limbus have cytoplasm that stains dark with smium (*D to the right*) *MC*, mesothelial cell 11-day- ld normal rat

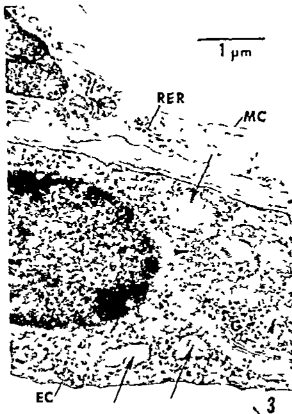


Fig 3 A portion of normal Reissner's membrane is more highly magnified here to better demonstrate the widely dilated cisternae of rough endoplasmic reticulum (*arrows*) filled with a granular substance typically found in the epithelial cells, (*EC*) at the juncture with the spiral limbus in very young rats A portion of the Golgi com plex (*G*) and large patches of glycogen particles (*arrowheads*) are present in the area of these cisternae The rela tive size of the cisternae can be determined by comparison with the more typical rough endoplasmic reticulum (*RER*) in the mesothelial cells (*MC*) above 11-day-old normal rat

The cells sometimes closely overlapped one another at their borders, but no junctional complexes occluded the free diffusion of peri-lymph around and between them Because of the general thinness of the cytoplasm and the absence of occluding intercellular junctional complexes, it was remarkable that numerous vesicles were present at the boundary mem-branes and intracellularly Most of these vesi-cles were uncoated, although a few coated vesicles were observed



Fig 4 Eleven day old treated rats show large patches of glycogen (*double arrows*) in some epithelial cells of Reissner's membrane particularly near the junction with the spiral limbus (*SL*) as illustrated here The cisternae of the rough endoplasmic reticulum of the epithelial cells are widely dilated and filled with a granular substance (*single arrows*) The cell (*D*) in the center of the field has more dense cytoplasm that stained dark with osmium *BL* basal lamina *G* Golgi complex *MC* mesothelial c 11 day old treated rat 4 doses 6-hydroxydopamine
Fig 5 The glycogen accumulates in aggregates (*d* arrows) in many of the epithelial cells of Reissner's membrane in treated rats by 17 days The aggregates are especially common in the lateral cell regions where

The basal surfaces of the mesothelial cells re irregular but the apical borders were gen lly smooth The depth of the basal undula ns largely determined the width of the fluid mpartment separating mesothelial and epi lial cell layers This compartment, which ntained a small amount of delicate fila ntous material was confluent with the peri nph of scala vestibuli through the intercel ar spaces

ithelial cells

e epithelial cells had abundant cytoplasm d were of rather uniform thickness except the nuclear region where the cells projected ghtly into the endolymphatic space (Fig 1) ie cells contained much rough endoplasmic ticulum and numerous mitochondria, espe- ally in the regions close to the intercellular fts The Golgi complex was extensive, etching from the perinuclear regions of each ll into the matrix near the clefts Coated sicles were commonly observed at the endo- mphatic surface of the cells and intracellu ly particularly near the lateral borders Numerous microvilli were present along the dolymphatic surface of each epithelial cell ie lateral cell margins were thrown into com cated plicae increasing the surface area of e cells enormously and causing the forma n of intercellular clefts of widely varying mensions (Fig 1) Each cleft was closed at e endolymphatic lumen by a zonula occlu ns a zonula adherens and then a desmo me commonly followed to complete the nctional complex The clefts were narrow at eir basal outlets and covered only by the ntinuous basal lamina Between the apical id the basal ends, the intercellular clefts were regular and dilatations were common

ish as de the organelles commonly found there The tercellular clefts are variously dilated at 17 days (*single rows*) with some narrowing evident Coated vesicles (V) are obvious at the endolymphatic surface of the ithelial cells (*EC*) as shown here but are uncommon sewhere *MC* mesothelial cell 17-day-old treated rat doses 6-hydroxydopamine

The basal surfaces of the cells often showed complex, plicated invaginations (Fig 1) The mouths of the invaginations were narrow and morphologically comparable to the basal out lets of the intercellular clefts

The juncture region of Reissner's membrane with the limbus showed certain morphological modifications in young and weanling rats (Fig 2) that tended to disappear by 28 days The epithelial cells of this region had rough endo- plasmic reticulum with greatly widened cis ternae containing a somewhat granular (but otherwise amorphous) substance (Figs 2 & 3) This same type of rough endoplasmic reti culum was present in the next few cells which extended over the surface of the spiral limbus Some of the latter cells had cytoplasm that stained dark with osmium Lysosomal bodies were common and the abundant mitochondria tended to be large

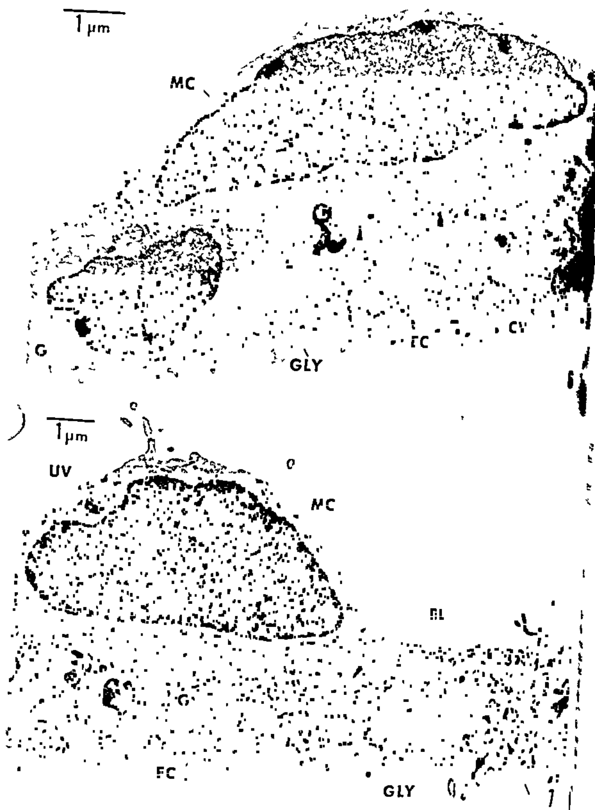
Glycogen particles ~150–300 Å in diameter were present in both cell layers of Reissner's membrane at its junction with the spiral limbus in 11 day old rats, but not in the older control animals The particles which were electron dense, were more numerous in the epithelial cell layer Sometimes, the accumulations of the polysaccharide particles were large (Fig 3) Occasionally, small clusters of the particles in the aggregates appeared to be confined within membranous structures, or remnants of them, that seemed collapsed or shrunken leav- ing clear zones in the surrounding matrix

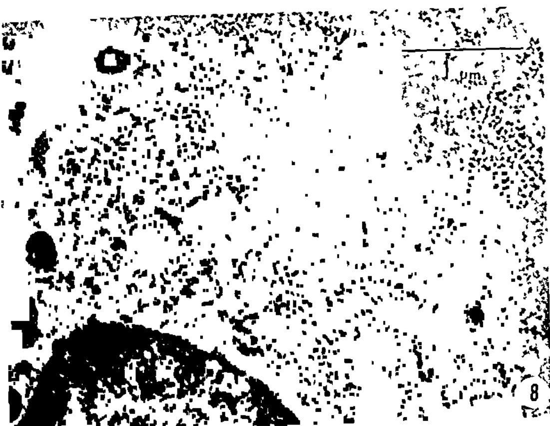
6 Hydroxydopamine Treated Rats

Reissner's membrane showed certain changes with respect to cytoplasmic inclusions or ganelles and intercellular spaces in treated ani mals 11–32 days of age These changes were more pronounced in the epithelial than in the mesothelial layer and tended to diminish with further time

Mesothelial cells

There appeared to be a decrease in the number of vesicles in treated rats up to and including


1 μm
MC
G
G
GLY
FC
CV
1 μm
UV
O
MC
BL
G
FC
GLY



8 Large amounts of glycogen remain in some of epithelial cells of Reissner's membrane even at 72 s The glycogen particles are of the β type, as shown e at higher magnification and are sometimes in linear string like arrangements (examples indicated by arrows) Many of the glycogen particles are found in the territory of the Golgi complex (*G*) and numerous mitochondria are nearby (*M*) *N* nucleus 72-day-old treated rat 12 doses 6-hydroxydopamine

days of age compared with controls (Figs 5, 6 & 7) Vesicles were, nevertheless, still numerous In contrast, the number of lysosomes increased Small patches of glycogen occasionally occurred in the cytoplasmic matrix The polysaccharide particles were variable in size (~150–300 Å in diameter) and electron dense Additionally, the basal surfaces of the cells showed fewer pronounced undulations during the early post natal period, the space between the mesothelial and epithelial cell layers was all but obliterated by 32 days (Figs 6 & 7) Except for the persistence of occasional small patches of glycogen (Fig 8), the mesothelial cells distal to the limbus juncture region resumed a more typical overall appearance by 72 days (Fig 9)

The mesothelial cells near the junction with the spiral limbus generally contained some gly-

6 Glycogen aggregates (*GLY*) almost fill the epithelial cells of the 32-day-old treated rat as shown here ated vesicles (*CV*) are found in numbers only at the dolymphatic surface Lysosomes (*L*) are numerous e intercellular clefts are uniformly narrow (*single ar ws*) and the space between the epithelial (*EC*) and sothelial (*MC*) cells is almost obliterated *G* Golgi mplex 32-day-old treated rat 4 doses 6-hydroxy pamine

7 Some of the glycogen in the epithelial cell (*EC*) own here is in aggregates (*GLY*) but elsewhere the rticles are more dispersed The intercellular clefts are rrow (*single arrow*) The basal lamina (*BL*) is distin ishable but the space between the two layers of Reiss r's membrane is much diminished in width Uncoated sicles (*UV*) are numerous in the mesothelial cell (*MC*) own here *G* Golgi complex 32-day old treated rat 4 es 6-hydroxydopamine

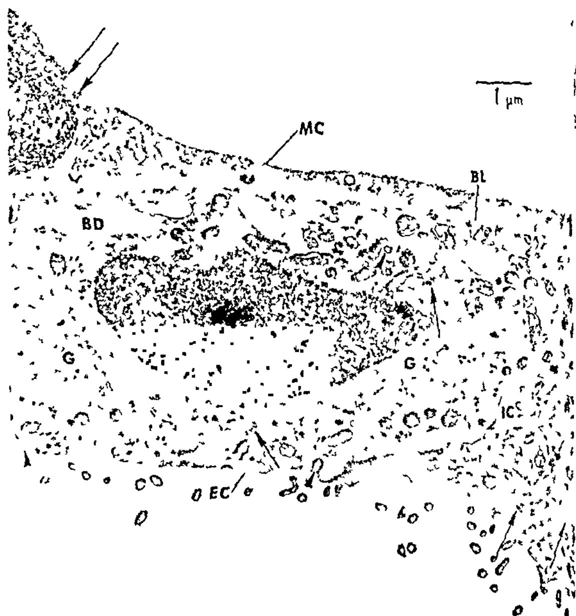
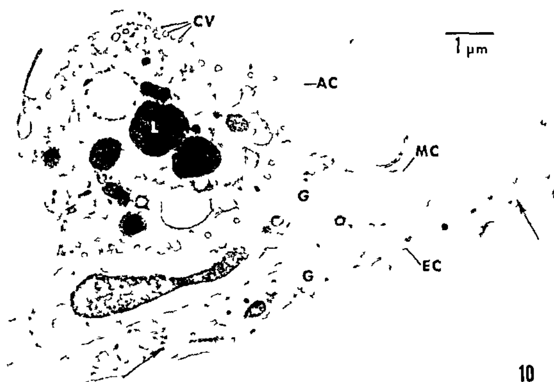


Fig 9 Variation in the amount of glycogen present occurred from one site to another in Reissner s membrane at 72 days The epithelial cell (*EC*) in the center of the field with more clear cytoplasm has widely dispersed small patches of glycogen particles (*arrows*) whereas the cell with more electron dense cytoplasm at lower right has slightly larger patches Glycogen particles (*arrows*) are also present in the mesothelial cell (*MC*) The intercellular clefts (*IC*) are variously dilated between the apical region of the junctional complex (*lower center right*) and the basal more constricted openings on the basal lamina (*BL*) The invaginations of the basal surfaces of the ep- (*BD*) Coated vesicle ounger treat- d rat 1° d-

6-hydroxydopamine

cogen at all ages, and polysaccharide particles were still present in this region at 72 days Moreover, the mitochondria became of giant size in the older animals with some of them measuring up to 1 µm in diameter compared with 0 2 to 0 4 µm at 11 days

There was an increased tendency for spheroidal structures resembling macrophages



10 The mesothel al layer of Re ssner s membrane in ited rats often showed adjunct cells (*AC*) as shown e Such cells conta ned numerous lysosomes (*L*) and e h ghly ves cular w th both coated (*CV*) and un es cles (*UV*) present In th s part cular sample of the tissue the intercellular clefts (*rrc w s*) of the epithel l cells (*EC*) are un formly narrow Glyc gen is present in small patches *G* Golgi complex *M* mes tl ell l cell 17 day-old treated rat 4 doses 6-hydroxydop m ne

y to become perched on top of closely related to the cells of the meso- al layer (Fig 10) These cells contained usual complement of organelles but were efly characterized by their many lysosomes d numerous vesicles of various sizes Many the vesicles were coated Uncoated vesicles large and sometimes contained a floc lent material lending a cystic appearance the cells

ithelial cells

three principal effects of the chemic il mpathectomy on the epithelial cells of e ssner's membrane were 1) glycogen ac mulation occurred 2) vesicles were numeri illy decreased and 3) the intercellul ir clefts narrowed and bec ime of rel itively uniform width

Glycogen iccumul ition was the first obvi ous effect observed in the epithelial cell l iyer with the polys icch iride particles evident in quantity by 11 d iys (Fig 4) The p irticles were electron dense and 150 300 Å in dia meter Polys icch iride particles increased numeric illy during further postnatal develop ment becoming accumul ited into large aggre gates in m ny of the epithelial cells by 17 d iys (Fig 5) At both 17 and 32 d iys the aggre gates were sometimes so l irge particularly in the l iteral cell regions that they displ iced the org inelles ordin irily found in the area (Figs 5-7) Glycogen p irticles were still pres ent in aggreg ites in m iny of the epithelial cel at 72 d iys (Fig 8) although other cells

a sharp decline in the number of these par ticles

Within the aggregates, the glycogen par ticles often had a tendency to group in linear arrangements or strings (Fig 8) Clusters of particles partially enclosed by what appeared to be collapsed membranous material were occasionally present

Coated vesicles were of typical numbers in the epithelial cells of the membrane in the 11 day old treated rats and the intercellular clefts were generally tortuous and dilated By 17 days however, the number of coated vesicles was visibly reduced The intercellular clefts varied in dimensions from one place to another (Fig 5) In some cases, the clefts were dis tinctly narrowed for a distance below the api cal junctional complexes and above the basal outlets, with more typical dilatations present in between In other instances the portions of the clefts visible in the micrograph were uniformly narrow resembling the condition commonly observed at 32 days of age (Figs 6 & 7) The apical junctional complexes and the basal openings retained their typical spatial relationships throughout An appreciable in crease in numbers of coated vesicles occurred in those epithelial cells showing decreased glycogen content at 72 days (Fig 9) but the clefts between all the epithelial cells of Reiss ner s membrane were more typically tortuous and variably dilated at this time

The epithelial cells at the juncture region with the spiral limbus contained much glyco gen at every age examined At 72 days the particles were sometimes dispersed in the cytoplasmic matrix but in other instances large aggregates of glycogen were present

DISCUSSION

Although numerous fluorescence and ultra structural investigations of the inner ear have supported the original findings of Spoendlin & Lichtensteiger (1966) that there are perivascu lar and blood vessel independent groups of adrenergic fibers in the inner ear (Terayama et al 1966 1977 Ross 1971 1973 Densert 1974) the functional significance of this exten sive sympathetic innervation has remained elusive Stimulation or surgical extirpation of various portions of the cervical sympathetic chain where adrenergic fibers of the eighth nerve are said to originate (Spoendlin & Lich tensteiger 1967, Terayama et al 1968) leads to changes in cochlear microphonic activity (Seymour & Tappin 1953 Beickert et al 1956), but the precise relationship of the ob served changes to manipulation of one or the other of the two groups of adrenergic fibers has not been demonstrated conclusively Neither does interruption of the cervical sym pathetic chain at the superior cervical ganglion result in more than equivocal changes in inner ear morphology after survival periods of up to 22 days (C G Wright personal communica tion)

Chemical sympathectomy with 6-hydroxy dopamine is a powerful tool to use in experi mental studies of long term possibly homeo static functions of the adrenergic nervous sys tem in the inner ear The drug can under ap propriate conditions of critical dosage (Thoe nen & Tranzer 1968 Jonsson & Sachs 1970 Tranzer 1971) cause reversible selective de struction of peripheral adrenergic nerve termi nals in the adult (Tranzer & Thoenen 1967 1968 Malmfors & Sachs 1968) and irreversi ble damage to postganglionic cell bodies in the newborn (Angeletti & Levi Montalcini 1970)

The technique is not without its pitfalls however An important time factor may exist (Saner & Thoenen 1971) and species differ ences are marked (Malmfors 1971) More over extensive destruction of the sympathetic system is essential if specific effects of cate cholamine deprivation are to be studied for even slight sparing of adrenergic terminals may be sufficient to maintain baseline activity of some target organs once supersensitivity to circulating catecholamine develops (see Lundberg 1971) The situation is complicated further in adult animals in which repair is rapid with complete anatomical regeneration

drenergic nerve terminals possible within months (Haeusler et al , 1969, Jonsson & hs 1970, de Champlain, 1971) although tional return occurs much earlier (Jons- & Sachs, 1972) The experimental proce- e of repeated injections of 6 hydroxydop- ne utilized in this study was planned tak- into account the disadvantages noted ve and was based upon methodology de- d earlier by Thoenen (1971)

hemical sympathectomy followed by ultra ctural study of inner ear morphology has n carried out previously in the newborn (Ross, 1972, Ross et al , 1974) and in the lt rabbit (Densert, 1975) In these in- ces, evidence of damage to the organ of ti was obtained The current series of ex- imental rats also showed structural altera- s in the organ of Corti, particularly in the ion of the inner hair cells, which are under her investigation Such evidence of delete- s morphological change is not conclusive ause it is subject to question on the basis possible artifact, or on grounds of some que, cell specific toxicity, as noted by Den- t (1975)

he positive finding of extensive glycogen umulation in Reissner's membrane follow- chemical sympathectomy cannot be attri- ed to artifact nor to a toxic action of the ine on the affected cells Such storage re- res synthetic activity and viable cells reover, study of animals sacrificed at vari- times clearly shows first a progression, n a regression, of glycogen accumulation related events (vesicle reduction and nar- ving of the intercellular clefts of the epi lial cells) between 11 and 72 days Partial urn of conditions to a more typical state ne time during the longest survival period y coincide with regeneration of at least ne of the peripheral adrenergic fibers This uld suggest that, even under our experi- method of repeated injection of new- animals with 6-hydroxydopamine, sys- ic destruction of peripheral adrenergic rons was not complete The overt signs of autonomic imbalance noted do indicate, how ever, that neuronal damage was extensive

Glycogenolysis in liver and some other cells is under the regulation of the adrenergic nerv- ous system through the intracellular, second messenger cAMP, as described by Sutherland and his colleagues (Sutherland, 1950, 1951, Sutherland & Rall, 1960, Robison et al , 1967, 1971, Sutherland et al , 1968, and many others) When circulating catecholamine levels are high, such cells are stimulated at specific β-adrenergic receptor sites (Robison et al , 1967) on their cell membranes, where binding of the amine occurs, to activate adenylate cyclase to catalyze conversion of ATP to cAMP This starts an "amplification cascade" which results in a breakdown of glycogen to glucose and in utilization of all glucose pre cursors to form free glucose Moreover, not only is glycogen catabolism stimulated, but glycogen synthesis is prevented Conversely, in the absence of appropriate levels of cate- cholamine, dissociation of the bound neuro- humor occurs This suppresses the cascading effects which lead to glycogen utilization but sets in motion enzyme systems promoting glycogen storage

It would seem apparent from the present re- sults that there must be similar β adrenergic receptors on the cell membranes of the epi- thelial cells of Reissner s membrane which ordinarily function (in the presence of suffi ciently high levels of catecholamine) to set in motion the appropriate enzyme systems to in- sure the availability of glucose and to prevent glycogen formation Under conditions of cate- cholamine deprivation, as in the present case of chemical sympathectomy, glycogen accu- mulation was favored By size (~150–300 Å) and by arrangement as single units, the glyco- gen was in the form of β particles

Because glycogen accumulation was not anticipated, even though catecholamines func tion in glycogen utilization and storage else- where in the body, no special techniques were used to preserve or to stain the polysaccharide particles Procedures ordinarily employed by

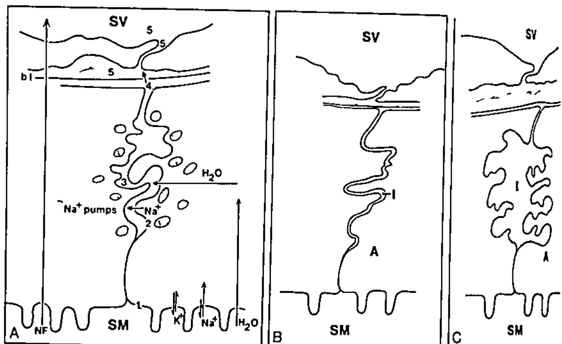


Fig 11 A Figure 11 A shows diagrammatically the sequence of events that could lead to net flow of Na^+/H_2O across the epithelial cells of Reissner s membrane from scala media (*SM*) into scala vestibuli (*SV*) This sequence is as follows

A Ions and water pass across the semipermeable membrane at the endolymphatic surface (*1*) into the cell passively, by diffusion

B Active pumping of Na^+ occurs across the lateral cell membranes (2) into the intercellular clefts (*3*) This causes an ionic gradient for Na^+ at the endolymphatic surface of the cell (*1*) and an osmotic gradient for H_2O across the cell and into the cleft (H_2O *arrows at right and center*)

C The movement of the H_2O into the cleft increases the hydrostatic pressure there forcing fluid across the basal opening (*4*) and the basal lamina (*bl*)

D The narrow basal opening and the basal lamina constitute a barrier to free flow of fluid impeding be[illegible] selectively restricting ion/fluid flow into [illegible]phatic space (*5*) scala vestibuli (*SV*)

E The net flow of solute/solvent is shown at left (*NF arrow*)

Fig 11 B C The degree of distension of the intercellular cleft is indicative of the functional state of the epithelial cells In (B) the cells are relatively inactive and are not moving solute/solvent in quantities sufficient to create a gradient for water across the cell (*A*) and into the intercellular cleft (*I*) In (C) the cells are very active [illegible] increased distension of the intercellular cleft (*I*) [illegible] the apical and basal ends of the cleft keep the[illegible] relationships in both cases *SM* scala media *SV* scala vestibuli

us for transmission electron microscopy, however, routinely include primary fixation with a glutaraldehyde mixture and post-fixation in osmium, which closely approximates the recommendations of Revel et al (1960) for preservation of glycogen for ultrastructural study

Why the epithelial cells mobilize glucose cannot be answered on the basis of the anatomical results alone, but narrowing of the intercellular clefts following glycogen accumulation in the treated animals is strong presumptive evidence that at least a portion of the glucose furnishes energy for a coupled solute-fluid transport system across the cells The concomitant decrease in the number of coated vesicles might or might not be related to the apparent reduction in such transport taking place across Reissner's membrane after chemical sympathectomy

By analogy to other cell systems that concentrate solutes in enclosed fluids, such as the gall bladder (Kaye et al , 1966), active transport of ions, such as Na^+, or other solutes into the intercellular clefts must normally [illegible] creating gradients both for the solutes and for water across the epithelial cells of Reissner's

⁻mbrane from the endolymphatic surface ; 11) The diffusion of water from the lolymph across the cells and into the clefts t would osmotically follow active pumping solute into the intercellular spaces would in- ease hydrostatic pressure in the clefts This uld, in turn, force the solutes and solvent uss the narrow basal openings of the clefts l across the basal lamina into the peri- nphatic compartment The width of the in cellular clefts, then, should reflect the level activity of the adjacent cells at any given oment The extreme narrowing of the clefts it was seen by 32 days after initial treatment th 6-hydroxydopamine would indicate that tive transport of solute into the spaces had tually ceased by that time

If the final passage of the water and solutes om the intercellular clefts into the perilym- atic space depends upon differences in hy- ostatic pressure and not upon active, selec- e transport, the fluids in the two compart- ents should normally be similar This would ggest that the epithelial cell layer of Reiss- r's membrane, like many other epithelial tis- es involved in fluid transport in vertebrates, ay have a coupled, energy dependent, $Na^+/$ $_2O$ transport system Regardless of the sol- e transported, however, the coupled move- ent of water suggests that Reissner's mem- ane may play an important role in helping to aintain ion and fluid balance in the inner ir, a function that in turn would appear to be bject to catecholamine regulation That the ortion of the membrane attached to the lim- us may be specialized for regeneration of amaged cells or for growth during early de- elopment has been suggested elsewhere ishiyama et al 1970, Johnsson 1971)

In light of the present results suggesting a oupled solute and fluid transport capability f the epithelial cells, it is interesting that hemical analytical studies indicate that trans- ort Na^+-K^+-ATPase activity in Reissner's ane is much lower than that of the stria lans (Kuijpers & Bonting, 1968, Mat insky & Thalmann, 1970) Matschinsky and Thalmann have pointed out that the amount of activity in the membrane is nevertheless as great as that found in many other secretory organs that pump cations It should be noted that these studies were carried out on the two-layered membrane, whereas our morphological findings would indicate that the active solute transport is confined to the epithelial layer of Reissner's membrane

With respect to the finding of extensive glycogen accumulation in the epithelial cells of Reissner's membrane following chemical sympathectomy, it is surprising that chemical analysis has shown the activities of the enzymes involved in glycolysis (Thalmann et al , 1970) and glycogenolysis (Matschinsky, 1970) to be relatively low (See also Thalmann, 1971) This finding must be reconciled with another, however, which demonstrated that phosphorylase a increased nine fold in Reissner's membrane within three minutes under the stress of ischemia, but only about three fold in the stria and one and one half times in the organ of Corti (Matschinsky & Thalmann, 1970) Phosphorylase a is the active form of the enzyme and is involved in glycogenolysis Unfortunately, histochemical studies of adenylate cyclase localization in the membranous labyrinth, which could shed some light on possible sites of catecholamine regulation on the epithelial cells, have yielded only equivocal results (Kerr & Schacht 1976, and personal communication) while recent biochemical analytical findings have indicated that the level of adenylate cyclase activity in Reissner's membrane is extremely low (Palaheimo & Thalmann, 1977) However, recent quantitative studies by Thalmann and Thalmann (1978) indicate a substantial activity of adenylate cyclase in Reissner's membrane (about 7 5 picomoles/mg dry weight/min)

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ZUSAMMENFASSUNG

Ratten wurden durch mehrfache Injektionen von 6-Hydroxydopamin wahrend der fruhen postnatalen Entwicklung chemisch sympathektomisiert Kontrollen vom gleichen Wurf blieben unbehandelt oder wurden nur mit Losungsmittel injiziert Innenohrgewebe von einer Serie behandelter und unbehandelter Ratten im Alter von 11

Membran, vor allem in den Epithelzellen nahe dem Limbus spirale Die Epithelzellen in diesem Gebiet besaßen auch rauhes endoplasmatisches Retikulum mit stark erweiterten Zisternen, die ein granulare Substanz enthielten Spater als 11 Tage war kein Glykogen vorhanden, und die Zisternen verkleinerten sich mit weiterer postnataler Entwicklung Die Epithelzellen der Membran in Kontrolltieren aller Altersstufen hatten interzellulare Spalten, die an der endolymphatischen Oberflache von Zonulae occludentes verschlossen waren, am basalen Ende eine enge Öffnung hatten und dazwischen verschie en weit waren „Coated vesicles" wurden haufig an apikalen und lateralen Zellgrenzen sowie intrazellular gefunden Behandelte Ratten hingegen zeigten eine Anreicherung von Glykogenpartikeln in den Epitheln, die zwischen dem 11 und 32 Tag anstieg und in einigen Zellen 72 Tage vorhielt Die Glykogenpartikel waren vom β-Typ, elektronendicht, ~150–300 Å im Durchmesser und waren oft zu großen Massen aggregiert, vor allem in den lateralen Zellregionen Gleichzeitig mit der Glykogenanreicherung nahm die Zahl der „coated vesicles" ab und die interzellularen Spalten verengten sich, bis sie nach 32 Tagen gleichmaßig eng waren Eine Zunahme der „coated vesicles" und eine Erweiterung der interzellularen Spalten wurde nach 72 Tagen beobachtet Die Ergebnisse werden dahingehend interpretiert, daß die Epithelzellen der Reißnerschen Membran die Fahigkeit haben zu einem energieabhangigen gekoppelten Ionen/Wasser-Transport, der normalerweise vom Glukosemetabolismus abhangig ist Es scheint daß der Glukosemetabolismus von Katecholaminen reguliert wird, was wiederum auf die Anwesenheit von β adrenergen Rezeptoren auf den Epithelzellen der Reißnerschen Membran schließen laßt

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MECHANICAL PROPERTIES OF BASILAR MEMBRANE

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tract A fresh basilar membrane has different mechan propert es in the radial and in the longitudinal direc s When pressure with a needle is exerted on the ilar membrane a narrow radially oriented strip is de ted The form of the deflection can be deduced from pathological consequences of the acoustic trauma as l The observed anisotrophy is a property of the vital mbrane and is disturbed by chemical and physical in nces and is lost post mortem The post mortem nges can explain the results obtained by von Bekesy ich d ffer from ours The physiological meaning of the chan cal properties of the basilar membrane is dis ssed here

ie theories and hypotheses about cochlear echanics as well as the physical and math natical models of the cochlea generally sume that the cochlear partition is a homo neous substance, dynamical properties of hich are similar to those of the rubber embrane However, the mode of the parti on vibrations on acoustic stimulation is not ependent only on the hydromechanical forces ansmitted to the fluids of the inner ear by apes but also on the sum of mechanical roperties of the individual structures of this norphologically very complicated organ Ac ording to the histological findings obtained up o date with respect to mechanics, cochlear partition can be considered to be an inho mogeneous body composed of components of differing rigidity, elasticity and plasticity

This paper deals with the mechanical prop erties of the basilar membrane The basilar membrane (BM) consists of the basic amorph ous substance and radially oriented fibres In pars tecta these fibres are arranged densely, one along the other, in pars pectinata the single layer is divided into two The upper layer which is near to the vestibular surface of the membrane is thin and compact, under it there is a layer of fibres grouped into bundles visible even under a light microscope Helmholtz took them for resonant strings

MATERIAL AND METHODS

Six week old guinea pigs weighing between 300–330 g were decapitated, their temporal bones removed and bullae opened After the removal of the bony cochlear shell, individual turns were exposed to gain access to the coch-lear partition Under a stereomicroscope using dethermal filter lighting cells of the Corti or gan were removed from the basilar membrane with a fine cotton wool brush During the dissection the tissues were wetted with the Ringer solution Above the decellularized BM a thin glass or metal needle with a rounded point of the diameter not exceeding 40 μm was fixed in Zeiss-Jena micromanipulator During the measurement the needle was moved perpendicular to the surface of the BM At a suitable angle of the incident dethermal filter light, the membrane and the changes of its surfaces were distinctly visible BM can be slightly stained with toluidine blue dye added to the Ringer solution, for the purpose of photo documentation, BM has to be stained more strongly



Fig 1 Microphotograph of the fresh basilar membrane deflected by the needle The deflection has a shape of a narrow radially oriented strip *Inset* Key *N* needle, *BM* basilar membrane, *L* spiral limbus

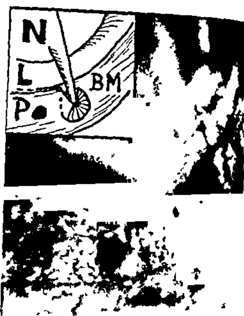


Fig 3 Microphotograph of the b[illegible] Key *N* needle, *L* spiral limbus *P* [illegible] *BM* basilar membrane

Besides the fresh cochleae, i.e those examined within 15 minutes of decapitation, the same method was used to examine the mechanical properties of BM in cochleae obtained from animals only after 24 hours after decapitation During the elapsed time the temporal bones were kept at a temperature of 5–10°C Temporal bones of other animals were fixed in a 5% and 10% formaldehyde solutions, in formaldehyde solution with soap addition in a 5% and 10% lysol solutions The reason for these examinations will be dealt with in the discussion

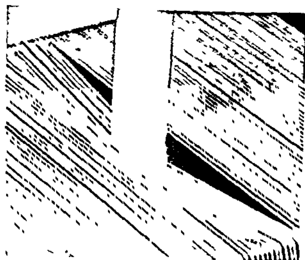


Fig 2 The [illegible]

RESULTS

By gradual pressure of the needle on the vestibular surface of the BM, there appeared in all turns of fresh cochleae the same form of membrane deformation A narrow, radially oriented strip was deflected Its length occupied the entire radial size of the BM i.e. from the spiral ligament up to lamina spiralis ossea, but its width was determined by the needle size. The thinner the needle the narrower was the deflected strip of the membrane The deformation did not spread along the partition in the longitudinal or spiral direction (see Fig 1) Under the needle a groove appeared in the form of parallel strings deflected by the pressure of a narrow object (see Fig 2)



g 4 Microphotograph of basilar membrane after the moval of Corti's organ cells After the displacement of µm the basilar membrane has its normal appearance ote the radial fibrous system

When the needle was removed, the BM as-umed its original position When a needle ressure was applied to cochleae that were not esh, but dissected up to 24 hours after the eath of the animal, a circular impression ap-eared, similar to that which appears when ressure is exerted on an elastic homogeneous membrane or on the skin on a soft tissue layer Similar circular deformation was found on he deflected basilar membrane in cochleae ixed with formaldehyde, with formaldehyde with soap addition, and on specimens depos-ted in lysol solution The impression in all he cases was always rounded, like a broad shallow crater If the needle shift was too big and exceeded the BM elasticity stress limit so that the BM broke, the perforation of the fixed BM was always circular (Fig 3) However, the orated hole in fresh membranes closed immediately after the removal of the needle so hat the rupture became invisible even when the membrane was examined at high magnification in a light microscope (Fig 4)

In specimens fixed with a 5% and 10% lysol solutions, the epithelium of the inner ear was considerably damaged, the basilar membrane was similarly deformed by a circular impres sion when pressure was exerted with a needle, yet the membrane was not so solid and it tore easily

DISCUSSION

In 1941, Georg von Bekesy published a paper in German on the elasticity of the cochlear partition The paper also described the form of the BM deformation caused by the pressure of a thin glass needle or a human hair In the apical turns of the human cochlea he found a circular depression, in the basal part of the cochlea near the stapes the deformation was elipse shaped with the longitudinal elipse axis corresponding to the longitudinal direction of the cochlea From his experiments, von Bekésy deduced that the BM had equal mechani cal properties both in the radial and in the longitudinal direction and that the BM be haved as a homogeneous membrane without any mechanical orientation He supposed that the eventual strengthening by the radial fibres is compensated for by the longitudinal elements of the tympanic layer (*tympanale Belegschicht*) which "in der Langsrichtung der Membran verlaufende Fasern zeigt" Hence the idea originated that the basilar membrane has no mechanical orientation and when de flected it behaves as a homogeneous membrane, e g a rubber membrane

It is evident from both the German original of the paper and its English translation (1948) that von Békesy used human material for his experiments In his book (1960), page 20, he writes "Fresh temporal bones are to be obtained from the prosector of a large hospital " It is evident that this material could not be fresh in the supravital sense, but it must have been several hours old, obtained most probably only the next day after death On

those basilar membranes that were examined up to 24 hours after death, we have also observed circular impressions under the needle The post-mortem changes after 24 hours damage the tissues of the Corti organ considerably, as described by Wersall et al in 1965 Such changes might be overlooked in a low magnification stereomicroscope, especially if the tissue had not been stained and the tympanal wall had been observed

The post-mortem changes increased during dissection when the specimens were immersed in tap water, as described by von Bekésy

Further, page 433 of the English translation (1960) reads "Measurements made over a period of two weeks showed no important changes for a temporal bone kept in Lysol solution at the temperature of 5°C ' The English translation is mistaken in the word *Lysol* The German original states that the bones were kept "in Lysoformlosung ' Lysoform was a trade mark name of a solution then widely used in Europe for external disinfection as well as for conservation purposes in anatomy It did not contain any Lysol Its formula reads Formalini 40% 100 ml, Spiriti saponis kalini 50 ml et aquae dest ad 1 000 ml That is why we have used in our experiments not only the formaldehyde solution but also one with soap addition conforming to the Lysoform formula Under the influence of the English version we have also examined the effect of the Lysol solution at 5% and 10% concentrations Von Bekésy's original papers never mentioned Lysol It can be seen that Lysol damages the tissues to a great extent, so that they soon become almost useless for experimental purposes However, even the formaldehyde solution fixation changes the chemical and physical properties of the tissues significantly, so that their mechanical properties become markedly different from those of the *in vivo* condition This was shown on fixed specimens When the BM is deflected by the needle it becomes considerably more rigid and the deformation appears as a relatively broad circular crater Furthermore, we have observed that the fixed membranes tear at smaller deflections than do the fresh ones It is not necessary to deal in detail with the effect of the fixation on individual tissue and cellular components It is a matter of common experience that the mechanical properties of biological objects change after having been fixed with formaldehyde

The experiments with fresh BMs unambiguously show their mechanical orientation The membrane can be presumed to deflect in narrow segments also when stimulated by noise This is confirmed also by our previous observations when the histological consequences of acoustic trauma were analysed After exposition to high intensity sounds, tiny perforations were found in the BM They were usually elongated, radially orientated and always of a very small diameter The BM perforations never joined, even when closely adjacent to one another These findings caused by intense noise certainly corroborate the experimental results obtained with needles

The high sensitivity of the hearing organ from the aspect of cochlear mechanics can be better explained by anisotropy of the BM For a deflection of the BM in a narrow radial strip a smaller volume of a liquid is necessary than for a greater deflection of a circular shape This is important for the auditory threshold A narrow deflection also facilitates better peripheral analysis of frequencies Although the original Helmholtz theory of the resonance of the BM strings tuned to the individual frequencies cannot be accepted, it seems that the mechanical orientation of the membrane facilitates its oscillation within very narrow and separated segments, similarly to hammers striking the dulcimer s strings Of course, the basilar membrane fibrils are not resonant strings but a limiting element which prevents the propagation of the deflection in the longitudinal direction in the basic plastic BM material of the cochlear partition The converse is also true—the plasticity of the basic material facilitates the oscillation of the membrane in narrow segments lying parallel with the radial

nls No mechanical importance of the mpanic layer was observed The observed sotropy is the property of the vital and pravital BM and it is easily damaged by emical and physical effects and is lost when e tissue dies

The above described experiment dealing th the mechanical properties of the basilar mbrane can be easily repeated if fresh ecimens are used and if physiological condi ns necessary for supravital tissues are re ected and observed

ZUSAMMENFASSUNG

frische Basilarmembran hat verschiedene mecha che Eigenschaften in der radialen und longitudinalen htung Durch den experimentellen Druck mit der del entsteht eine langliche Auslenkung die parallel zu n Rad alfasern der Basilarmembran orientiert ist Die rm der Deviation der Membran kann man auch aus den hologischen Folgen des akustischen Trauma ableiten Die beobachtete Anisotrophie ist nur die Eigenschaft der Vital Membran und sie wurde postmortem sowie auch durch die chemischen und physikalischen Einflusse ver loren Post mortem Anderungen konnen auch die Dif ferenz zwischen unseren und von Bekesy s Befunden auf klaren Die mechanischen Eigenschaften der Basilarmem bran sind für die Physiologie des Gehors bedeutungsvoll

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REDUCTION OF THE ENDOCOCHLEAR POTENTIAL BY THE NEW "LOOP" DIURETIC, BUMETANIDE

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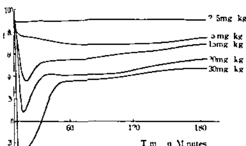
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Abstract The effect of bumetanide upon the endocochlear potential (EP) was examined in 46 guinea pigs The EP was reduced with dosages of 5 mg/kg or more The maximum depression of the EP (reduction to −30 to −40 mV) was obtained at a dosage of 30 mg/kg The recovery of the potential was incomplete at any dosage within three hours and the response pattern of the EP to bumetanide was similar to that to ethacrynic acid The present experiments revealed that bumetanide, by weight has a stronger ototoxic potency than the other loop diuretics—furosemide and ethacrynic acid However the diuretic effect of 1 mg bumetanide is equivalent to 40 to 60 mg furosemide or ethacrynic acid Therefore the relative ototoxic potency of bumetanide is much smaller suggesting that from a clinical standpoint bumetanide is much safer than the other loop diuretics

Bumetanide (3 *n*-butylamino-4-phenoxy-5-sulfamoylbenzoic acid) (BUM) is a new "loop" diureitc whose primary diuretic action is exerted in the ascending limb of the loop of Henle, similar to the other "loop" diuretics ethacrynic acid (EA) and furosemide (FU) (Feit, 1971, Østergaard et al, 1972) EA and FU are known to have ototoxic side effects and have been shown to have a profound influence upon the endocochlear potential (EP) (Prazma et al 1972, Bosher et al, 1973, Kusakari et al, 1977) Their main site of action in the cochlea is considered to be the stria vascularis (Quick & Duvall, 1970, Bosher et al, 1973) We could find only one report in the literature suggesting possible ototoxicity in man—a transient hearing loss following the administration of 1 mg BUM (Asano et al, 1974) Bourke (1976) reported three cases in which deafness occurred following the administration of FU, but the clinically equivalent diuretic dosage of BUM (one fortieth that of FU by weight) showed no evidence of ototoxicity Brown (1976) reported that BUM exerts pronounced effects upon the EP in the cat Since the guinea pig is widely used in experimental studies, and since "loop" diuretics show wide variations in diuretic effects among various species, it seemed relevant to determine the action of BUM upon the EP in this animal

MATERIAL AND METHODS

Forty-six albino guinea pigs weighing 250 to 350 g with normal Preyer pinna reflex were used Tracheostomy was done under pentobarbital anesthesia (28 mg/kg) and the animal was artificially respirated after i p administration of succinylcholine chloride The left bulla tympanica was opened wide via a ventrolateral approach The electrode (a glass micropipette with a tip diameter of about 2 µm filled with 150 mM KCl, and connected to a Grass [illegible] d c microelectrode preamplifier by means of a chlorided silver wire) was advanced via the round window, through the basilar membrane and into scala media The EP of +80 to +90 mV (with reference to the potential of scala tympani) was recorded with a pen oscillograph, the indifferent electrode was placed on the exposed neck muscles BUM solution in dosages from 2 5 to 40 mg/kg was administered over a period of 3 minutes through an indwelling catheter in the right jugular vein The EKG was monitored throughout the ex-



1 The effect of intravenously applied bumetanide on the endocochlear potential in the guinea pig The drug was administered at the indicated dosages over a period of 3 min via an indwelling catheter in the right jugular vein

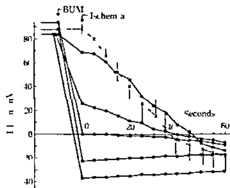


Fig 3 Effect of ischemia upon the EP of bumetanide intoxicated ears (●—●) The dotted curve indicates the decline rate of the EP in untreated control ears (mean +S D , N=21 Adapted from Kusakari et al 1977)

...nment and the body temperature was maintained at about 37°C

RESULTS

The initial magnitude of the EP in the present experiment was 84 8±4 8 mV (means ±S D , N=46) No effect upon the EP was seen in 2 animals given 2 5 mg/kg of BUM, however, at 5 mg/kg and above, a dose dependent reduction of the EP was obtained Typical response patterns are shown in Fig 1 After a latent period of one to two minutes, the EP began to decline rapidly, reaching its lowest level within 6 to 15 min (except in the case of 5 mg/kg, with which the EP declined on a much slower time scale) The maximally reduced levels were 72 0±4 4 mV (n=3), 54 0±13 1 mV (N=3), 30 7±37 4 mV (n=12), −0 8±29 4 mV (n=11), −25 0±7 5 mV (n=4), −31 9±10 6 mV (n=7), and −35 8±9 4 mV (n=4) for dosages of 5, 10, 15, 20, 25, 30, and 40 mg/kg, respectively (Fig 2) After the maximally reduced level was reached, a biphasic recovery set in (i e an initial rapid recovery period, followed by a slow progressive one), however, in no case was complete recovery seen within the maximum observation period of three hours, with the higher dosages (30 and 40 mg/kg) the EP recovered only to +30 to +40 mV during this time Although a great deal of variation in response between animals was seen at dosages of 15 and 20 mg/kg, the responses were quite reproducible with the higher and lower dosages

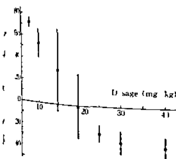


Fig 2 The effect of intravenously applied bumetanide upon the endocochlear potential expressed as a function of drug dose Data points represent means ±S D

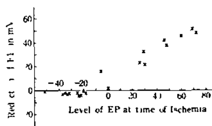


Fig 4 The effect of bumetanide intoxication upon the ischemic decline of the endocochlear potential Ischemia was induced by sectioning the aorta when the endocochlear potential had reached its maximally reduced level in response to bumetanide (*abscissa*) The *ordinate* represents the reduction of the potential from the preischemic level after 35 sec of ischemia

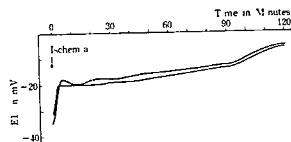


Fig 5 The effect of ischemia upon the endocochlear potential of ears severely intoxicated by bumetanide illustrating the reduction of negativity which occurs when the level of the endocochlear potential is reduced to below −15 to −20 mV in response to the drug

In a second series of 20 animals, the aorta was sectioned when the EP had reached its maximally reduced level in response to various dosages of BUM As shown in Figs 3 and 4, the ischemic decline rate of the EP in these pre intoxicated animals decreased with the degree of primary damage induced by the drug, however, when the maximally reduced level was lower than about −20 mV, ischemia caused a decrease in negativity In the 2 cases shown in Fig 5, the EP began to rise within 10 sec after the onset of ischemia, and increased from a level of primary damage of about −30 and −35 mV, respectively, to about −20 mV within 10 minutes After remaining at this level for 30 to 40 minutes, the EP gradually increased toward the baseline A reduction of negativity was also seen in superimposed respiratory hypoxia The EP returned to the preasphyxic level upon reventilation (Fig 6)

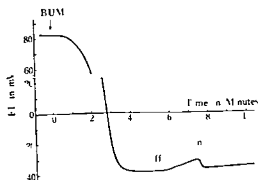


Fig 6 The effect of respiratory hypoxia (off) and subsequent reventilation (on) upon the endocochlear potential of ears severely intoxicated with bumetanide

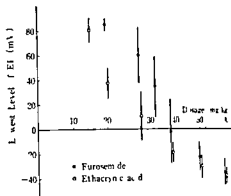


Fig 7 The effect of intravenously appl ed e harm

1977)

DISCUSSION

EA, FU, and BUM are potent diuretics whic act primarily in the ascending limb of the lo of Henle Although there are minor diffe ences, these drugs share a common mecha ism of action in the nephron (Davies et al 1974, Olesen et al , 1973 Kim et al 19 Stein et al , 1968, Østergaard et al 19 There are many clinical reports on the otot icity of EA (Schneider & Becker, 1966 Pi et al , 1969), and FU (Schwarz et al 19 Electrophysiological, biochemical and m phological studies on the inner ear suggest t their primary site of action is the stria vas laris and that the EP is the primary electr parameter involved (Prazma et al 197 sher et al , 1973, Quick & Duvall 1970 sakari et al , 1977) Although both drugs duce the EP, the response pattern is quite ferent—namely, the recovery of the EP i complete within 2 hours after administratio EA, whereas the EP returns to its ori level within one or two hours even with l dosages of FU (Kusakari et al 1977) Br (1976) studied the effects of BUM upon c lear potentials in the cat and showed tha duration of ototoxic action of BUM is si to that of FU and less than that of EA ever, the results obtained in the present in the guinea pig indicate that the resp pattern of the EP to BUM more close

mbles that of EA Species differences have ng been recognized in the case of EA and U

Fig 7 shows the maximally reduced level of e EP induced by various dosages of EA and U in the guinea pig (Kusakari et al 1977) In e comparison of the three diuretics shown in ies 2 and 7 it is evident that on a weight sis BUM is the most potent ototoxic agent, llowed by EA and by FU However, 1 mg of UM exerts a diuretic effect equivalent to 40 60 mg FU (Olesen et al , 1973 Davies et al)74) and EA is reported to have an absolute otency almost identical to or slightly greater an that of FU (Laragh, 1967) Therefore hen the diruetic dose–response relations and nical dosages of these three drugs are taken ito consideration the relative ototoxic po ncy of BUM is far lower than that of the ther two drugs The present study indicates iat although BUM is potentially ototoxic, the ossibility of deafness occurring in patients re iving therapeutic dosages of the drug is re iote because of its relatively smaller ototoxic otency per clinical dose

Kuijpers (1969) proposed that the normal EP onsists of two components a large positive lectrogenic potential (100 to 120 mV), thought) be produced by active K^+ transport in the tria vascularis and a small negative potential hought to be a K^+ diffusion potential (−20 to 40 mV) Although there is some disagree nent concerning the exact nature of the two omponents (e g Honrubia et al , 1976) a ual nature of the EP seems to be supported y most investigators

Bosher et al (1973) proposed that the de line of the EP in the early stages of EA in oxication is due to the abolition of the positive ecretion potential consistent with this pro osition is the behavior of the high energy hosphates of the stria vascularis in EA intoxi ation which suggests a pronounced inhibi on of energy utilization (Thalmann et al 1973 Kusakari et al , 1977) However, there appear to be moderate effects upon energy eneration as well

Another line of evidence, originally thought to represent an additional reflection of a re duced metabolic rate, is the fact that the ischemic decline rate of the EP predamaged by EA is much lower than that in non intoxicated animals As shown in Figs 3 and 4 analogous results were obtained in the present studies with BUM, however, since ouabain exerts a much stronger inhibition of the ischemic de cline of high energy phosphates but a dis tinctly lesser reduction of the ischemic decline rate of the EP as compared with EA, we no longer feel that this is a valid indicator of a reduced energy use rate (Kusakari et al , 1977)

When the EP was reduced to a level lower than −15 to −20 mV by BUM superimposed ischemia produced an elevation of the poten tial The presence of this anoxia sensitive negative potential was also observed in the case of EA and FU (Thalmann et al , 1973, Sellick & Johnstone 1974, Kusakari et al , 1977) An analogous pattern in EA intoxicated animals was reported in the utricular and the ampullar EP (Sellick & Johnstone 1974, Ku sakari & Thalmann 1976) it is not clear at this point whether this is a normally existing potential unmasked by EA or FU administra tion or an abnormal potential which appears only in intoxication with loop diuretics

Bosher et al (1973) suggested that the initial decline of the EP in EA intoxication is due to inhibition of strial enzymes, followed by a more prolonged phase of altered membrane permeability On the basis of *in vivo* and *in vitro* experiments with ouabain which indi cate remarkably similar effects upon the EP and upon strial Na^+K^+ ATPase, Kuijpers (1969) postulated that this enzyme is involved in the generation of the EP Since the effects of EA upon the EP are similar to those pro- duced by ouabain the assumption that the ef fects of EA are also due to interference with this enzyme did not seem unreasonable How ever, recent studies (Kusakari et al 1977, Paloheimo & Thalmann 1976) dem that the inhibition of strial Na^+K^+-ATP

EA *in vitro* requires concentrations 500 times higher than those required to inhibit the EP by local administration These results are in agreement with the report of Kuijpers & Wilberts (1976) on the differential effects of EA and ouabain upon strial $Na^{+}K^{+}$-ATPase activity

By contrast, the inhibition curves of the EP and strial adenylate cyclase activity *in vitro* with respect to EA are very similar Marked effects of EA upon adenylate cyclase and cyclic AMP, and a possible role of these two substances in ion transport and permeability have been described in other organs (Ebel, 1974, Tria et al , 1974) Although the effects of EA upon strial adenylate cyclase are consistent with the proposition that interference with this enzyme may be the mechanism by which the EP is affected (Ahlstrom et al , 1975, Paloheimo & Thalmann, 1976), further studies are necessary to decide whether or not there is a causal relationship

ACKNOWLEDGEMENT

We express our thanks to Professor R Thalmann Washington University for his help in the preparation of this manuscript

ZUSAMMENFASSUNG

Der Effekt von Bumetanid auf das endocochleare Potential (EP) wurde bei 46 Meerschweinchen untersucht Dosen von 5 mg/Kg oder daruber reduzierten das EP Eine maximale Senkung des EP (Verminderung auf −30 bis −40 mV) wurde mit einer Dosis von 30 mg/Kg erzielt Die Erholung des EP war bei allen Dosen unvollständig innerhalb der Beobachtungszeit von drei Stunden Die Reaktion des EP auf Bumetanid war ahnlich wie diejenige auf Ethacrynsaure Die Experimente lassen erkennen daß Bumethanid (bezogen auf Gewicht) eine starkere ototoxische Wirkung hat als die anderen loop Diuretika Furosemid und Ethacrynsaure Jedoch der diuretische Effekt von 1 mg Bumetanid ist derselbe wie derjenige von 40 bis 60 mg Furosemid und Ethacrynsaure Deshalb ist die relative ototoxische Wirkung von Bumetanid geringer was bedeuten wurde daß vom klinischen Standpunkte aus gesehen Bumetanid viel harmloser ist als die anderen loop Diuretika

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THE DEVELOPMENT OF HAIR CELLS IN THE EMBRYONIC CHICK'S BASILAR PAPILLA

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Abstract During the 7th to 21st (hatching) days hair cells of the embryonic chick transform from an undifferentiated epithelium into cylindrically shaped tall hair cells (THCs) pitcher shaped short hair cells (SHCs) or intermediate *hair cells that share structural characteristics of the first* two By the 11th day hair cell types are unambiguous Hairs (stereocilia and a kinocilium) were first identified on the 7th day and resembled the adult pattern the 13th The nucleus occupies relatively less volume as hair cells increase in size becoming positioned centrally in THCs and basally in SHCs Nucleoli which are prominent throughout development remain conspicuous even in newly hatched chicks The cuticular cone begins to form the 11th day By the 10th day sensory nerve endings synapse on the bases of the HCs which by the 11th day develop synaptic bars Although efferent neurites were in evidence as early as the 11th day synaptic contacts and their cisterns were identified by the 19th day but may form earlier Supporting cells transform from cylindrically to flask shaped cells with constricted necks and may secrete at least a portion of the tectorial membrane

In recent years, the adult avian basilar papilla, the counterpart of the mammalian organ of *Corti* has been examined ultrastructurally in the pigeon (Cordier 1964*a b*, Rosenhall, 1971, Takasaka & Smith, 1971), and cock (Janke et al , 1969) Within the basilar papilla of the *auditory lagena, there are three types of hair* cells tall, short, and intermediate (Takasaka & Smith, 1971) These sensory cells are distinguishable on the basis of size, shape, stereociliary length shape of cuticular plate or cone, and possibly innervation pattern By comparison, the developing basilar papilla has largely been overlooked In fact the chick's cultured otocyst has served doubly as a model system for avian hair cell differentiation (Friedmann, 1959*a b* 1968 1969) and also for the action of ototoxic antibiotics (Friedmann & Bird, 1961, Friedmann 1965) In term[...] cultured otocysts, however hair cells [...]parently do not complete differentiation, only one of the three hair cell types is ide[...]fiable (Friedmann, 1969) The underlying [...]tors responsible for the arrested differentia[...] are unknown Recently, Hirokawa (197[...] [...]ported on aspects of hair cell developme[...] normal embryos and those treated with β-[...]garotoxin In the present study we follo[...] the differentiation of hair cells in the [...]bryonic chick s basilar papilla in order to c[...]pare the normally developing lagena to a[...] and to cultured otocysts [2]

MATERIALS AND METHODS

For this study more than 250 White Le[...] eggs were used Groups of 1–4 dozen [...] were incubated in a thermostatically [...]trolled incubator that was set at 38±2 C [...] were turned clockwise and countercl[...] at least twice a day during the incubation [...]riod Prior to decapitation, the embryos [...] staged according to Hamburger and Ha[...]ton's scheme (1951) Eggs from the 7th to [...] (hatching) days were used primarily, a sm[...] number within 4 days after hatching

Immediately after the lagenar tissue was [...]posed, it was placed in cold (4–8°C) glut[...]dehyde (3–3 5%) buffered in 0 1 M sod[...]

[1] In partial fulfillment of the Master of Science degr[...]
[2] This represents an expansion of material prese[...] the Southeast Electron Microscopic Society USA [...]min & Cohen 1977 Siegel Fermin & Cohen 197[...]

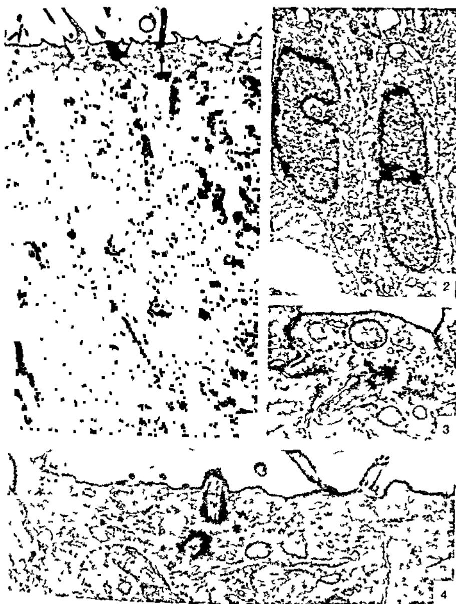


Fig 1 Apical region of b... ve cytc... ior to ... e vac... Apical extensions the future microvilli and stereocilia are not distinguishable though a kinocilium (arrow) is present 7th day ×9000

Fig 2 Invaginated nuclei at apical end of basilar papilla possibly indicating high synthetic activities 7th day ×6000

Fig 3 End vacuole or ciliary body of Golgi complex 7th day ×29000

Fig 4 Diplosome in apex Nascent kinocilium is emerging from surface Microfilaments strengthen junctions 7th day ×29000

...acodylate and containing 5 mM Ca^{2+}, and less ...equently in glutaraldehyde (2%) buffered in ...1 M sodium phosphate, both at pH 7 2–7 4 ...After the lagena was freed from the temporal ...one it was post fixed in buffered 1% osmium tetroxide, pH 7 2–7 4, for 1–2 hours The tissue was then dehydrated in either a graded series of acetone or reagent alcohol (50–100%), embedded in epoxy (Araldite 502) and sectioned with Sorvall s MT 1 ultramicro-

Table I *Dimensional changes of hair cells (expressed in microns)*

Hair cell type	Days of incubation								
	7th day Undif epithelium	11th day		14th day		19th day		21st day (newly hatched)	
		THC	SHC	THC	SHC	THC	SHC	THC	SHC
Hair cell length	9–13	12–14	7–8	10–16	8–12	15–23	9–13 6	17 24	10-14
Hair cell width	2 9–5 2	4–4 3	4 6–5 7	4–6	4 5–6 5	4–4.5	4–5	4 2–4.5	[illegible]
Nucleus long axis	2 5–6 1	4 7–6 5	4–4 5	5–8	4 3–5	5–8	4–5	3 8–7 7	[illegible]
Nucleus short axis	1 7–3 7	2 7–4 7	4–4 5	3–4 5	3 1–3 8	3–4 4	3–3 8	2 2–4 2	[illegible]
Cuticular cone	0	0 2–0 5		0 4–0 8		1 5–2 5		3.5–4.5	[illegible]
Tallest stereocilia	0 8–1 2	1 5–2 0		2 5–2 8		3–5 0		3 5–6.5	

tome The silver-gray sections were stained first with uranyl acetate (Watson, 1958) and then counterstained with lead citrate (Reynolds, 1963) Sections were examined and photographed with a Zeiss 95-2 transmission electron microscope at magnifications ranging from 1 900 to 21 000 and enlarged optically

RESULTS

We used the anatomical terminology that was recommended by Takasaka & Smith (1971), which is more appropriate for the avian ear, as was the term "cuticular cone" instead of "cuticular plate" (Jørgensen, 1971) The central basilar papilla was used predominantly in the present study because of the presence of all three hair cell types

7th day (Stage 31)

Because the pseudostratified epithelial cells of the basilar papilla appear uniform, presumptive hair and supporting cells can only be identified by relative positions, i e , apical or basal, rather than by specific structural features or densities (Fig 1) Cell divisions are infrequent but have not yet ceased as evidenced by mitosing cells particularly the more apical cells Many nuclei are deeply invaginated (Fig 2), possibly indicating high synthetic activity Centrioles of recently divided cells are centrally positioned prior to their apical ascent A diplosome forming the kinocilium's basal body often displays an [illegible] vacuole or ciliary body of the Golgi compl[illegible] (Fig 3) A rudimentary kinocilium (Fig [illegible] consisting of an axonemal skeleton that is [illegible]ally arranged in a 8+2 pattern rather than [illegible] more common 9+2 of most other cilia exten[illegible] outward 0 7–1 2 μm from the apical surface [illegible] almost every cell The other apical proj[illegible]tions will form stereocilia or microvilli but [illegible] distinction between them is not presently p[illegible]sible (Fig 1 and Table I) Tight junctions a[illegible] desmosomes zipper the apices of adja[illegible] cells together The tight junctions are stre[illegible]ened by bundles of microfilaments (Fig 4) [illegible]low these junctions, there are numerous ex[illegible] cellular spaces, which are largest at the ba[illegible] regions of the basilar papilla and give a sp[illegible] appearance There are also intracell[illegible] spaces or empty vacuoles (Fig 1) that [illegible] sometimes limited by a membrane Althou[illegible] resembling fixation artifacts these e[illegible] vacuoles had once been filled with lipids t[illegible] were extracted during tissue processing t[illegible] become less frequent subsequently The b[illegible]lar membrane forms on the buttressed base[illegible] presumptive supporting cells by an und[illegible] coating of amorphous extracellular mate[illegible] that is re-enforced with collagen fibers (F[illegible] 25) The nascent basilar membrane is rid[illegible] with empty spaces through which many s[illegible] neurites have already coursed the neurites [illegible]parently do not wedge between crevices [illegible] adjoining cells Although they have reach[illegible] midway within the basilar papilla the neur[illegible]



Fig 5 First presumptive hair cell It rests on superior fibrocartilaginous plate, close to columnar cells, and is identifiable by slightly denser cytoplasm Microtubules traverse the cell's long axis A forked stereocilium extends from the surface In presumptive supporting cell, satellite bodies encircle kinocilium's basal body 8th day ×43 000

Fig 6 Midbody The microtubules (approx 230 Å in diameter), in this case 52, are remnants of the mitotic spindle 8th day ×45 000

have not yet established synaptic contacts upon hair cells

The cellular cytoplasms are quite dense and richly endowed with organelles that are evenly distributed For example, polysomes become more numerous as differentiation proceeds, contributing later to the increased cytoplasmic density of hair cells Chains of ribosomes, both free and attached to membranes, surround sacs of proteinaceous material. Later, once distinction between hair and supporting cells is possible, it becomes evident that these sacs are confined to supporting cells

Compared with later stages of development, the mitochondrial population is small In thin section, only 6–15 mitochondria are counted, each approximately 1 0–2 5 μm in length and 0 3–0 5 μm in diameter Nestled within the mitochondrial cristae are opaque granules (500–700 Å) that are probably calcium deposits and remain as a consistent feature thereafter The first traces of the tectorial membrane appear as a thin wisp of material hovering over the cellular projections (Fig 1).

8th day (Stage 34)

Differentiation of hair cells begins first at the superior fibrocartilaginous plate where the presumptive tall hair cells are forming and then proceeds laterally across the basilar membrane towards the inferior fibrocartilaginous plate where presumptive short hair cells will form. In the former, there are several cells (14 μm in length) which are distinguishable

from the other by their slightly denser cytoplasm and are considered as nascent hair cells (Fig 5) Occasionally rudimentary stereocilia sprout from their apices However, their lack of other cytological landmarks prevents their unequivocable identification Junctional complexes are also found in the superior sulcus, the transitional zone between the future columnar and hair cells

In both hair and supporting cells, 10 to 15 granular satellite bodies (500 Å) encircle each kinocilium's basal body (Fig 5) Mitochondria and microtubules are slightly polarized and aligned in parallel (paraxially) to the cell's long axis Although the mitochondrial population has remained constant, the number of microtubules in the supranuclear region increased A few secretory granules are dispersed within the cytoplasm (Fig 5) The last few cells are completing division as evidenced by a midbody (Fig 6), a remnant of the mitotic spindle

9th day (Stage 35)

As hair cells become progressively denser, they now are more readily distinguishable from supporting cells, hair cell types, however, are not yet recognizable Stereocilia grow outward from the hair cells and small microvilli sprout from the supporting cells Although cytoplasmic organelles of hair cells begin to migrate, mainly to the supranuclear region, they do not congregate immediately beneath the stereocilia but instead circumscribe the area where formation of the cuticular cone will commence shortly

10th day (Stage 36)

Afferent nerves establish the first synaptic contacts on the bases of hair cells At the site of contact, the basal membrane becomes denser

11th day (Stage 37)

Hair and supporting cells are now readily distinguishable from each other hair cells are shorter but denser, and in contrast, the supporting cells are longer (about 2x) but lig[illegible] Two hair cell types are now identifiable T[illegible] elongated tall hair cells (Fig 7) narrow sl[illegible]ly into a neck whereas the stubby short h[illegible] cells (Fig 8) display a truncated neck wi[illegible] flared collar At their flat apical ends both [illegible] and short hair cells buckle slightly into [illegible]siently domed surfaces perhaps [illegible] lateral expansion that will be particularly p[illegible]minent in short hair cells

The hair cells sprout stereocilia tha[illegible] crease in length in a stepwise fashion [illegible] 2 5 μm), and, though not fully grown are [illegible] readily distinguishable from the shorter [illegible] narrower microvilli of the adjacent suppor[illegible] cells (Figs 7 and 8) The stem of each ste[illegible]cilium is filled with a filamentous core that [illegible]rows into a short dense neck through whic[illegible] single rootlet extends downward to anch[illegible] into the nascent cuticular cone The root[illegible] may indeed be the microtubules seen earlie[illegible] the 8th day, and are now redeployed at [illegible] 11th to associate with young stereocilia [illegible]fore the cuticular cone has started to form [illegible] kinocilium is presumably nudged laterally [illegible] emerging stereocilia until it rests eccentrica[illegible] within the cell but medially to the cell's ce[illegible] and faces the superior fibrocartilaginous pl[illegible] in the supporting cell, which lacks stereoc[illegible] the kinocilium is centrally positioned

In both tall and short hair cells as the [illegible]ticular cone begins to form it appears a[illegible] granular accretion that displaces organelle[illegible] it collects beneath the apical surface (Fig [illegible] Multivesicular bodies appear but are conf[illegible] to the supranuclear region as are secret[illegible] granules (0 4 μm in diameter)

A synaptic bar (Fig 19) forms within [illegible] base at the site opposite the afferent term[illegible] that established contact the previous d[illegible] Vesicles about 350 A encircle the syna[illegible] bars, and some also contact the plasma m[illegible]brane Tall hair cells are innervated bef[illegible] short Efferent neurites, identified on the b[illegible] of cytoplasmic density and presence of v[illegible]cles, appear inside the basilar papilla but [illegible] not yet establish synaptic contact (Fig 22)



Fig 7 Na ent tall hair cells ly ng on ledge of superior fibrocartilaginous plate (SFP) Because differen at on ad an e from the SFP to the nferior f brocartilaginous pla e hese ce s are darker han the former 11th day ×5000

Fig 8 Short hair cells Stereoc lia are shorter but mo e numerous than n tall hai cells Ap cal surfaces are domed 11th day ×6000

Fig 9 Tall hair cells Cylindrical shape s maintained as ell lengthen 14th day ×6000

Fig 10 Short hair cell Spout is forming eliminating the prev ous symmetry 14th day ×6000

Fig 11 Tall hai cells Cytoplasm s packed with free ribosomes organelles are polarized in both hair and supporting cells 20 days ×2500

Fig 12 Short hair cell Pitche shape resembles the adul s 3 days after hatching ×5000

Fig 13 M tochondrion embedded within cuticular cone 4 days after ha ching ×5000

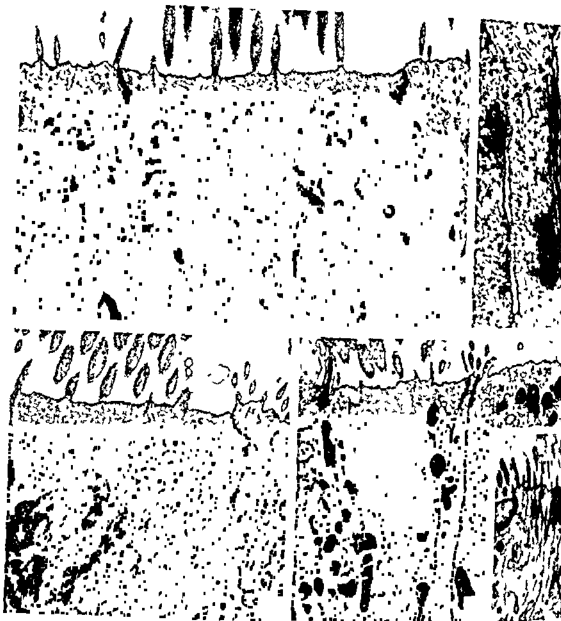


Fig 14 Nascent cuticular cone of tall hair cell with full complement of synthetic organelles beneath it Subsurface cistern runs paraxially (arrow) Supporting cells' cytoplasms are becoming progressively less dense, mitochondria are smaller than those of adjacent hair cell 14th day ×19000

Fig 15 Surface cistern flanked by mitochondria Subsurface cisterns, which are located apically or centrally, are identifiable earlier than the subsynaptic 14th day ×35000

Fig 16 Cuticular cone in tall hair cell Growth place by numerou

Fig 17 Rootlets extend downward through cone 3 days hatching ×14000

Fig 18 Microtubules extending up into microvillar 3 days after hatching ×19000

The supporting cells are cylindrically shaped. Secretory granules, Golgi complexes, endoplasmic reticula (cisternal and tubular), and microtubules associated with the microvilli, become conspicuous Mitochondria are scarce, and in cross-section have small diameters than those in the adjacent hair ce Supporting cells are permanently attached the tectorial membrane by means of fibr material that seems to extend from the

icrovilli and also from the transient kino- This thin fibrillar material arches over shorter stereocilia of the hair cells and a cupulla Only the tips of the tallest are embedded in the lower surface the tectorial membrane, the shorter stereo- if attached at all, are embedded more ly and withdraw during fixation

1 day (Stage 38)

orming as early as the 7th day (Fig 4), junc- complexes which are strengthened in- nally by bundles of cytoplasmic microfila- ents but are not always apparent because of e plane of sectioning, join the apical ends of e hair and supporting cells to each other ig 27) Immediately below the apical ends, ng (1–2 μm) tight junctions join hair and sup- orting cells together, and also adjacent sup- orting cells

day (Stage 40)

he spout of the short hair cell, which will be- ome pitcher shaped, grows laterally, giving e first inkling of the mature appearance (Fig)) tall hair cells do not change their cylin- ical appearance (Fig 9) Cytoplasmic or- anelles which prior to the 11th day appear ndomly distributed, congregate primarily neath the nascent cuticular plate In the stal end of the basilar papilla, these de elopmental events lag behind

The subsurface cistern is a membrane sys- m (1–2 μm in length) that lies in parallel to hair cell s lateral plasma membrane (Figs and 15) and is similar in appearance to the naptic cistern Ribosomes are usually ttached to the cytoplasmic face and mito hondria congregate nearby Although both ilateral and unilateral subsurface cisterns ave been identified, it is not known whether he latter is a normal occurrence or is instead ue to the plane of sectioning

In the supporting cell, the transient kino- ilium disappears, the cisternal population that ad collected in the apex peaks before its dis- harge and the neck is invaded by Golgi com plexes At their bases, coiled rough endo plasmic reticula (rER) also make their appearance The tectorial membrane reaches the climax of its formation

16th day (Stage 42)

From the 11th–16th days (Fig 16), the cuticular cone increases in size by growing downward and displacing the underlying organelles Large numbers of microtubules are paraxially aligned and seem to converge on the cuticular cone Multivesicular bodies remain closely associated with the cuticular cone

In the supporting cells (Fig 16), the cisternal population of rER is already being replaced by large number of Golgi complexes Occasionally, the basal body of the transient kinocilium is still present The cytoplasmic density is steadily declining

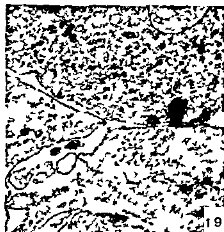
17th day (Stage 43)

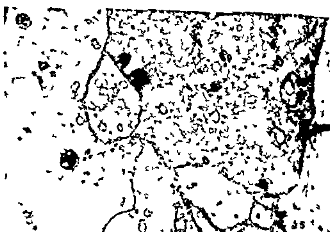
In hair cells, organelles still remain beneath the developing cuticular cone Efferent nerve fibers have not yet reached the bases by hair cells In the bases of the supporting cells, the coiled rER is being replaced by fragmented rER, and at the apex or neck of supporting cells, Golgi are receding but are more numerous than cisternal rER

19th day (Stage 45)

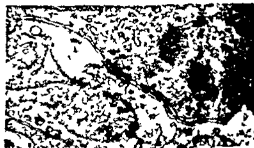
In the hair cells organelles are still congregated below the incomplete cuticular cone (Fig 11), which now extends down as far as 2 5 μm (Table I), and the stereocilia with long rootlets reach up as much as 4 5 μm The cuticular cone often touches the lateral membrane and points towards the inferior fibrocartilaginous plate Occasionally, stray microtubules are embedded within the cuticular cone

We identified as many as 4 synaptic bars in a single hair cell with multiple endings A single afferent terminal may exhibit two syn aptic bars which in some instances may be


19








22



μm apart or in others almost fused together sharing the same vesicles Efferent ter- 's now begin to establish synaptic con- with hair cells (Fig 23), but they are rela- ely infrequent at this time The first sub- naptic cistern also appears concurrently h the efferent contact and directly faces it *ne efferent synapse is characterized by its* allel membrane that is also filled with dense terial (Fig 24)

In the neck of the supporting cells Golgi omplexes, polarized paraxially, have suc- ded the *majority of previous organelles* bules traverse the clear cytoplasm and at the base of the microvilli Meanwhile, their bases, abundant *amounts of frag-* rER are now present instead of circu- conglomerates of tubular rER, a pattern hat is observed in the newly hatched chick

0th–21st days (Stage 46)
nd hatching

By hatching the hair cells (Fig 11 and 12) ex- ibit the same morphological characteristics as hose observed in adult chickens, except that he cytoplasm is densely packed with free ibosomes In all three hair cell types, the uticular cones have almost reached their adult size (Fig 17) Stereocilia, with their rootlets anchored firmly into the cuticular cone, extend upward sometimes up to 6 μm from the short hair cells (Table I) The organelles are no longer congregated beneath the cuticular cone and are now dispersed, having lost their previous polarity A stray mitochondrion, microtubules and vesicles are sometimes *embedded within the cuticular* cone (Fig 13) Few Golgi complexes and rER are found in the supranuclear region Up to 30 mitochondria can now be counted in a thin section, having doubled in number from earlier stages *Although some mitochondria are peri-*nuclearly positioned, they are mainly grouped in either supra- and infranuclear regions of the *hair cells, since now, the nucleus lies very* close to the lateral walls of the elongated tall hair cells (Table I) The nucleus has also increased its diameter slightly (Table I)

Afferent nerve endings more frequently synapse on tall than on short hair cells (Figs 20 and 21), the converse is true for efferent (Fig 24) However, synaptogenesis continues at least for a short period, which accounts for the increased number of afferent and efferent terminals after hatching Even though the afferent synapse is irregularly contoured, the pre- and post synaptic membranes remain in register and the synaptic cleft is filled with dense material A group of axons lose their myelin sheaths as they enter through the habenula perforata (Fig 26)

In the neck of supporting cells, the cytoplasm is *clear* and the organelles have *almost* completely disappeared except for a few Golgi complexes, which are now paraxially polarized The kinocilium's basal body has also disappeared in most cells, though there are exceptions (Fig 28) The supporting cell s apex is constricted to about 0 5 μm, which is one fourth the width at the 11th day The number of satellite bodies has decreased by two-thirds, their symmetry is also lost At their bases, fragmented rER still remains but in smaller amounts Microtubules extend into microvillar bases (Fig 18) but the microvilli contain microfilaments

Fig 19 Earliest afferent terminal on tall hair cell Synaptic bar encircled by vesicles hovers over synapse 11th day ×29000

Fig 20 Multiple afferent terminals on tall hair cells here are two synaptic bars in one terminal and one in the 3 days after hatching ×19000

Fig 21 Afferent terminal in tall hair cell shortly before hing Two synaptic bars that are sharing vesicles face synapse 20th day ×19000

Fig 22 Efferent neurite penetrating into basilar papilla close to basilar membrane and not yet synapsing upon hair cells 11th day ×14000

Fig 23 The first indication of an efferent synapse which is recognizable by the subsynaptic cistern Terminal contains a few scattered vesicles 19th day ×29000

Fig 24 Efferent ending They are more frequently found on bases of *short* hair cells and are also usually larger The terminal contains clear vesicles Subsynaptic cistern is filled with a dense material and ribosomes adhere to the cytoplasmic face 3 days after hatching ×24000



Fig 25 Nascent basilar membrane ...

Fig 26 Habenula perforata Axons penetrate through the hole in basilar membrane and then course tortuously between supporting cells The basilar membrane rests on the ledge of the superior fibrocartilaginous plate The darker, vesiculated fibers are presumed to be ... 3 days after hatching ×5 000

Fig 28 Diplosome in supporting cell Diplosomes ... at this developmental stage Microvilli fan out from ... constricted apex 3 days after hatching ×14 000

DISCUSSION

Differentiation of hair cells

We determined the developmental sequence of auditory hair cells in the embryonic chick at the fine structural level The epithelial cells of the basilar papilla undergo terminal mitotic divisions between the 7th and 8th days of incubation (Fig. 1) Cellular differentiation begins immediately after the cessation of mitosis but does not occur simultaneously throughout the lagena (Fig. 6). Instead, there are two developmental gradients the proximal portion differentiates before the distal and the supe... portion matures before the inferior; a para... pattern occurs along a basal to apical gradi... in the coiled mammalian cochlea (Rub... 1967) For example, the denser nascent h... cells first become distinguishable from t... paler supporting cells between the 8th and 9... days at the superior fibrocartilaginous pl... (Fig. 5), followed by the cells in the infer... fibrocartilaginous plate, as also reported ea... lier by Rebollo and Rodriguez (1964) and R... bollo and Casas (1964). Friedmann (19... demonstrated that explanted otocysts, thoug...

agging slightly, achieved equivalent develop ment by the 12th day of culture

Under light microscopy, the basilar papilla of 11 day embryos resembles that of adults, and by the 13th day gives the impression on cellular shapes that histogenesis has largely ceased and maturation is completed (Rebollo & Casas, 1964) However, when specimens of the same age are examined ultra structurally, it is obvious that the nascent hair cells are actively engaged in the formation of more conspicuous specialized features, such as the stereocilia, and the cuticular cone (Figs 14 16, 17) The three hair cell types, i e, tall intermediate and short, become distinguishable the 11th day (Figs 7 and 8) Intermediate hair cells are not discussed further because they share characteristics common to both tall and short hair cells Hirokawa (1977) was able to identify the embryonic chick's tall and short hair cells by the 14th day but apparently did not sample between the 10th–14th days when the major transformations are occurring Held (1927) illustrated two hair cell types (pp 493, 495) in the embryos of the chick and 16 day pigeon which he described as "zylindrisch and "kegelformig" but did not mention when they first became distinguishable Kikuchi & Hilding (1965*a*) demonstrated that murine outer and inner hair cells are identifiable at birth

Kinocilia closely resemble the structure of motile cilia They terminate in basal bodies (Figs 3 and 4) that are believed to arise from centrioles after the final mitotic divisions (Kikuchi & Hilding, 1965*a*) Avian kinocilia may serve a sensory function, for, along with the tallest stereocilia, they are embedded into the lower surface of the tectorial membrane In the embryonic chick, a single kinocilium is centrally positioned in each presumptive hair and supporting cell of the undifferentiated epithelium (Fig 1) The kinocilia are structurally identical to those in pigeons (Rosenhall 1971, Takasaka & Smith, 1971) and young adult cocks (Janke et al , 1969) and are taller than adjoining stereocilia, unlike rudimentary kinocilia of terminally cultured otocysts (Friedmann, 1967, 1969) In birds, kinocilia, and their basal bodies in hair cells but not supporting cells (Fig 8) remain permanently after maturation (Takasaka & Smith, 1971) In mammals, cochlear kinocilia are transient structures that disappear shortly after hair cells mature, but their basal bodies remain (Kikuchi & Hilding, 1965*a*), except in the cat (Spoendlin, 1966), vestibular kinocilia, however, are retained in adults (Engstrom et al , 1962) Although kinociliary loss is interpreted as a feature of more advanced ears (Janke et al , 1969, Rosenhall, 1971), its significance is uncertain because auditory sensitivity is seemingly unaffected

All the stereocilia of a hair bundle begin to form concurrently but then grow at unequal rates, the stepwise differences in height are evident as early as the 11th day (Figs 7 and 8) Stereocilia are taller in tall hair cells than in short (Table I) Rosenhall (1971) has pointed out that the bird s hair cells are not arranged in rows as are mammalian outer and inner hair cells, but instead lie in a mosaic pattern Nonetheless, stereocilia and kinocilia, when present, are morphologically polarized throughout the auditory lagena (Rosenhall, 1971, Takasaka & Smith, 1971) and cochlea (Wersall & Lundquist, 1966)

The cuticular cone, a droplet of granular-amorphous protein that also contains PAS positive material (Rebollo & Rodriquez, 1964), anchors the stereocilia The shapes of cuticular cones differ after the 14th day (Figs 9–13, 14, 16, 17) In tall hair cells, they are narrower and deeper, where in short hair cells they are wider and shallower (Table I) As the cuticular cone grows downward, stereocilia grow upward (Table I), suggesting a relationship between the two The rootlets of tall stereocilia are longer than those of short and are anchored in the thicker portion of the cuticular cone The stereociliary rootlets sometimes traverse the entire length of the cuticular cone (Fig 17), but in our observations do not extend into the cytoplasm as often occurs in

mammalian cells (Kimura, 1975) Occasionally, a mitochondrion (Fig 13) is embedded within the cuticular cone

The subsurface cistern is evident by the 14th day (Figs 14 and 15) As implied its proximity to adjoining supporting cells, it may serve as a metabolic link between them In mammals the subsurface cisterns are highly fenestrated and face a fluid space, suggesting that they take up material By comparison, the subsynaptic cistern faces an efferent nerve that is the same size and closely follows its contours throughout much of its extent (Fig 24) Kimura (1975) has suggested that the subsynaptic cistern may limit the spread of transmitter substance, but conversely may also facilitate efferent nerve activity In adult mammals, these two membrane systems are directly connected, which implies that each influences the other (Kimura, 1975) Henkart et al (1976) proposed that subsurface cisterns may couple intracellular activity to electrical activity We have not yet determined whether the subsynaptic cistern, which appears the 19th day, forms from an expanding subsurface cistern as it descends into the cell s basal end or is induced by contact of the efferent, or, once formed, attracts the efferent nerve Hirokawa (1977) located prospective efferent contacts as early as the 14th day on the basis of subsynaptic cisterns At present we cannot completely reconcile this temporal difference, though he may have sampled the more precocious proximal end

In the embryonic chick, the differentiating hair cells, which display a granular cast, are considerably denser than those of the adult pigeon (Rosenhall, 1971, Takasaka & Smith, 1971) and young adult cock (Janke et al, 1969), attesting to the high level of synthetic activity, the invaginated nuclei of the differentiating epithelium (Fig 2) may presage these events As hair cells lengthen the nuclei increase slightly in diameter (Table I), nucleoli (Figs 7–13) are more prominent than adults In tall hair cells the nuclear membrane closely approaches the sides and in short hair cells the basal end (Figs 7–12) As a result the c
plasmic organelles migrate or are displa
Sher (1971) demonstrated similar
migrations during postnatal maturation o
murine cochlea From the 11th da
hatching, the supranuclear regions of
and tall hair cells contain an abundanc
rough endoplasmic reticula, secretory gra
and several multivesicular bodies the
being lysosomal derivatives (Novikof
Holtzman, 1976) In contrast other orga
steadily decline in number, especially i
infranuclear region of tall hair cells w
Golgi apparatuses and multivesicular b
are rarely seen after hatching when this s
was ending By comparison mitochondr
come more numerous as hatching (21st
nears and also shortly thereafter The su
sion of organelles undoubtedly represent
transition between diminishing synthetic
emerging functional activities Audition
ever, apparently precedes structural ma
in the chick but not the mouse For inst
Vanzulli & Garcia Austt (1963) rec
microphonic potentials as early as the
day Moreover, Grier et al (1967) de
strated that embryonic chicks respon
sounds as early as the 12th day and in
can be imprinted prenatally to patte
sounds In the murine cochlea hearin
measured by Preyer's reflex, cochlear p
tials and 8th nerve action potentials app
9 to 14 days postnatally when the orga
Corti has largely completed maturation (A
& Ruben, 1963)

Innervation

According to Knowlton (1967) neurites p
trate inside the basilar papilla as early a
4th day, but by the 7th day, when this s
began, have not yet established synapse
hair cells (Fig 25) Although afferent n
endings first contact the hair cell bases w
are displaying slight membrane thickening
the 10th day, it is not certain whether the
tions are functional By the 11th day the
branes are denser, and synaptic bars the

ence of afferent synapses (Cordier, 1964*a* nd *b*, Smith 1967), first appear presynapti ally (Fig 19) The vesicles that surround the ynaptic bars do not yet encircle them as sym etrically as they will regularly by the 13th ay

In the embryonic chick, the innervation pat rn on hair cell types corresponds to that on e adult pigeon (Rosenhall, 1971, Takasaka & mith 1971) Nerve fibers lose their myelin eaths as they pass through the habenula per- rata and advance toward the bases of the air cells (Fig 26) Takasaka & Smith (1971) aced the course of afferent nerve fibers in igeons and demonstrated that 90% did not ranch at their terminals, only a few (2–5%) xhibit several branches In the embryonic hick by comparison, Fermin & Cohen (un- ublished) showed that afferent nerves branch ore frequently and then also arborize in the cinity of hair cells The hair cells usually ceive more than one afferent synaptic ter inal (Fig 20) but we do not know whether ese multiple terminals represent mono- or olyneuronal innervation For instance, tall air cells receive one to five afferent synaptic ontacts which are usually small boutonic ter inals (Fig 21) but occasionally receive large outonic, short hair cells receive one to two ontacts which are usually large boutonic riedmann (1969) demonstrated large cresen c contacts in cultured otocysts but these ap- ear to be vestibular rather than lagenar hair ells In birds the synaptic contacts of the udi tory lagena are restricted to the basal end f hair cells (Cordier, 1964*a* and *b*, Takasaka & Smith, 1971), whereas in the mammalian ochlea they frequently synapse laterally Smith 1967)

Although efferent synapses may form ear- ler as suggested by Hirokawa (1977), we ound the first convincing evidence two days rior to hatching (Fig 23), when the hair cells nd associated structures of the basilar papilla re almost terminally differentiated, efferent eurites may penetrate into the basilar papilla by the 11th day (Fig 22) The efferent ter- minals contacting tall hair cells are fewer and smaller than those synapsing upon short cells Although they may be closely apposed, ef ferent nerve endings apparently do not syn apse upon afferent nerves, a pattern that is common for inner but not outer hair cells of many mammals (Spoendlin, 1973)

Kikuchi & Hilding (1965*a*) reported that in the mouse efferent nerve fibers are first ob- served by the 8th–9th days postnatally when the organ of Corti closely resembles that of the adult They (1965*b*) also showed by using a strain of hereditarily deaf mice (Shaker-1) that the hair cells begin to degenerate shortly after the time that efferent innervation fails to form, implying that the developing otocyst re quires efferent innervation to complete dif ferentiation and to function fully Moreover, according to Friedmann (1959 1965, 1969) the absence of efferent innervation may be one reason why hair cells in cultured otocysts did not complete differentiation but instead loosely resembled tall hair cells of 15–17 day embryos, the afferent nerve supply remained intact In contrast, Van De Water (1976) re ported that removal of the statoacoustic gang lion complex of murine cultured otocysts at different critical testational periods did not adversely affect differentiation Nonetheless, it can be argued that the nerves had already imparted their influence before the excision and that in this instance afferent rather than efferent nerves are the more important Hiro- kawa (1977) demonstrated that the embryonic chick's hair cells developed normally but were slightly smaller even when both the afferent and efferent nerve supplies were lethally damaged with the nerve poison B bungaro- toxin, suggesting that hair cell differentiation, including formation of synaptic bars, synaptic membrane thickenings and densities, and cis terns does not require neural influences

Supporting cells

During development, as hair cells become denser and acquire new structures, the sup- porting cells become lighter and change their

shape by transforming from long cylindrical cells to ones that are highly constricted at the apex (Fig 18) and greatly expanded at the bases, an arrangement that holds the hair cells in place and confers sufficient rigidity for stimulation Nonetheless, there is the possibility that supporting cells serve additional functions (Siegel et al , 1977)

In birds, it is known that six supporting cells, each tightly coupled to the adjoining one, completely encircle every hair cell at its upper or apical end (Takasaka & Smith, 1971) Supporting and hair cells are also coupled to each other by tight junctions and desmosomes Orr (1975) showed the early formation of junctional complexes in cultured chick's otocysts The junctional complexes ensure that the endolymphatic and perilymphatic fluids, which differ markedly in composition, do not mix by flowing between the hair and supporting cells The same junctional configuration binds hair cells and the reticular lamina in the mammalian cochlea (Iurato, 1967) Dohlman (1967) has suggested that homologous cells in the membranous labyrinth, the plana semilunata, may also serve a metabolic role in nourishment and maintenance of vestibular hair cells More recently, Cohen et al (1977) demonstrated that supporting cells contain higher glycogen levels than the adjacent hair cells, which lose glycogen as differentiation nears completion Janke et al (1969) also suggested that the supporting cells may be secretory, for they noticed that their microvilli were ensnared in the filamentous strands of the overlying tectorial membrane This putative secretory function is strongly implied by the congregation of cellular machinery in the apices of the cells (Figs 13–18) The sacs of proteinaceous material apparently discharge their contents, for these organelles disappear after the foregoing period of intese synthetic activity The cytoplasm then becomes clear and loses its abundant population of organelles (Figs 11–18) Kikuchi & Hilding (1965*a*) observed that the mouse's hair and supporting cells have transient kinocilia that are sh-d shortly after birth In the embryonic chick the kinocilia of the supporting cells are usually shed around the 14th day, but the basal bodies some[illegible] remain until after hatching (Fig 28)

Satellite bodies, putative complexes of nucleic acids that may be related to localized synthetic activities of microtubules (Berns et al , 1977), encircle the basal body (Figs 1[illegible] 27) but become less numerous and [illegible] as hatching nears, probably corresponding to the generally diminished synthetic activities Though present in both hair and supporting cells, they are more conspicuous in the [illegible] *cytoplasm of the supporting cells*

ZUSAMMENFASSUNG

[illegible]

ersten Arten zeigen Am 11 Tag sind die Haarzel[illegible] Typen unzweideutig Haare (mehrere Stereocilia [illegible] ein Kinocilium) wurden erst am 7 Tag identifiziert [illegible] am 13 Tag glichen sie schon denen der Erwachsenen D[illegible] Nucleus nimmt verhaltnismaßig weniger Raum e[illegible] wahrend die Haarzellen sich vergroßern um sich sch[illegible] lich im Mittelpunkt der HHZ und am unteren Teil [illegible] KHZ zu befinden Die Nucleoli die während der E[illegible] wicklung sehr deutlich zu erkennen sind bleiben [illegible] [illegible] H hnchen Der R[illegible]

[illegible]

nten am 11 Tag sichtbar waren wurden synapt[illegible] Beruhrungen und deren Zistern erst am 19 Tag [illegible] aber die Stutzzellen verwandeln sich von zylinder[illegible] Zellen zu flaschenartigen Zellen mit verengerten H[illegible] und diese mochten teilweise die Membrana [illegible] ausscheiden

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CSF TOTAL PROTEIN NORMAL VALUES

A Re appraisal and Discussion of its Value in Diagnosis of Acoustic Neuromas

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Abstract A reference material of total cerebrospinal fluid protein (CSF protein) from 53 men and 45 women is presented Lowry s Folin phenol method for determining CSF protein has been used unchanged in this laboratory since 1964 with normal values ranging from 0 2 to 0 4 g/l [illegible]

[illegible] determining CSF protein in diagnosis of acoustic neu[illegible]

[illegible] showed both statistically as well as clinically significant increased protein but these tumours can be easily diagnosed by other means A more detailed determination of CSF protein is discussed

In 1951 Lowry et al published their study on the measurement of very low concentrations of proteins with the use of Folin and Ciocalteu s phenol reagent after pre treatment of the protein with an alkaline copper solution Since then this method, or modifications of it, under names as the Lowry–, the Folin–Lowry, the Folin–Lowry–Ciocalteu's method etc has been widely accepted for determination of proteins in dilute fluids In 1956 Lous et al after a careful evaluation of the method for determination of total protein in cerebrospinal fluid (CSF) recommended it as ' a simple, accurate and highly satisfactory routine method" The method was introduced in this department of clinical chemistry in 1964 and has been used unchanged since then At the time of its introduction no available normal values for the method existed and therefore the values 0 20–0 40 g/l based on an earlier turbidimetric method (Merritt & Fremont Smith, 1938) were used

Due to the lack of a reference material of our own, and due to an increasing feeling from the clinicians that the reference values were too low (Bjørum et al , 1972), the collection of a reference material was undertaken

MATERIAL

98 persons, 53 men and 45 women, in the age range 18–72 years, were included, 75 were psychiatric patients and 23 orthopedic patients who, due to minor disorders, were to be operated upon under spinal anaesthesia The patients were neurologically and medically normal according to the recommendations of The Committee of Reference Values of The Scandinavian Society for Clinical Chemistry and Clinical Physiology (1975) None of the patients received medication in the form of streptomycin sulfanilamide, salicylates, acetylsalicylic acid, phenacetin or psychotropic drugs apart from diazepam 10–20 mg/day, which were given as a sedative or hypnotic, when indicated

Lumbar puncture technique

The lumbar puncture was performed between 8 and 9 a m after the patients had been kept fasting and recumbent in bed for the preceding 10 hours The patients were placed in

Table I *Comparison of the present Folin–Lowry method with the biuret method*

Specimen	Dilution	Protein concentration g×l⁻¹ Biuret method Measured	Biuret method Calculated	Folin–Lowry method Measured
Seronorm® batch 127	undiluted	67		
	1 100		0 67	0 72
	1 200		0 34	0 41
	1 333		0 20	0 23
Serum pool	undiluted	69		
	1 100		0 69	0 72
	1 200		0 35	0 40
	1 333		0 21	0 26

lateral position and local anaesthesia was induced by lidocaine norepinephrine A CSF specimen of totally 10 ml was obtained by fractionated sampling Only clear and colourless CSF was used for analysis (Bech et al , 1978)

Protein determination

The modification of the Lowry method used for protein determination is that of Daughaday et al (1952) scaled down to use only half the volumes

Lous et al (1956) and Cannon et al (1974) have described and evaluated this method in detail, and only the major principles are described here

Two reactions are involved, (1) by addition of Cu^{2+} ions in alkaline solution the protein is complexed as in the biuret reaction (2) The Cu protein complex reduces the added Folin–Ciocalteu reagent (polyphosphomolybdic and polyphosphotungstic acid) to the deeply coloured molybdenum- and tungsten blue In proteins—especially the amino acids—tyrosine and trytophan give rise to an extra staining, adding to that produced by the Cu–protein complex Certain drugs such as streptomycin, salicylates, acetylic salicylic acid, phenacetin and chloropromazine also cause staining Moreover it has been shown that IgG exhibit 20% higher absorbance (on a mass basis) in the Folin–Lowry method than does albumin (Rieder, 1974, Cannon et al , 1974) Because of the variation in chromogenicity of various proteins, the use of diluted serum as standard for measuring CSF proteins as proposed by Cannon et al (1974) will standardize the error due to this difference

Double determinations were performed on 200 μl CSF taken from the third ml CSF As standard was used a serum pool with a total protein mass concentration of 70 g/l determined by the Kjeldahl method diluted 1 100 with 154 NaCl to 0 70 g/l No corrections for non protein material, according to Daughaday et al (1952) 0 06 g/l, was made

The analytical variation (precision) between days shows a standard deviation of 0 010 g/l The samples were in the range 0 20–1 20 g/l with a mean value of 0 49 g/l This gives a coefficient of variation of 2%

In an attempt to estimate the analytical bias (the accuracy) of the method as it is run in this laboratory, a short re evaluation was performed in connection with this study The protein solutions used as controls in the serum protein determination was analysed in dilutions of 1 100, 1 200 and 1 333 It was (1) Seronorm® batch no 127 with a protein co-

Table II *Protein concentration of cerebrospinal fluid control (lot 4p154) values of the present Folin–Lowry method compared with the declared values*

Method	Protein concentration g×l⁻¹
	Declared values ($\bar{x}\pm 2s$)
Turbidimetry trichloroacetic acid[a]	0 80±0 12
Turbidimetry trichloroacetic acid[b]	0 85±0 15
	Measured value ($\bar{x}\pm 2s$)
Folin–Lowry (present method)	0 82±0 04

[a] Dupont aca®
[b] Henry et al (1956)

Table III *CSF total protein in a reference material of 98 persons grouped according to sex and age*

Sex	Age (years)	Number of subjects	CSF protein g×l⁻¹ Median	Range	Signif of diff
♀	18–40	17	0 39	0 23–0 69	
♂	18–40	27	0 50	0 26–0 96	P 0 02
♀ ♂	18–40	44	0 44	0 23–0 96	
♀	40–59	19	0 43	0 28–1 06	
♂	40–59	18	0 53	0 35–0 98	P<0 05
♀ ♂	40–59	37	0 49	0 28–1 06	
♀	60–72	9	0 45	0 31–0 70	
♂	60–63	8	0.53	0 41–0 87	P 0 11
♀ ♂	60–72	17	0 49	0 31–0 87	
♀	18–72	45	0 42	0 23–1 06	
♂	18–63	53	0.53	0 26–0 98	P 0 001
♀ ♂	18–72	98	0 47	0 23–1 06	

centration of 67 g/l, and (2) a pooled serum, used as control on the Autoanalyser® SMA 6/60 with a protein concentration of 69 g/l, both determined by the biuret reaction This method is standardized with human serum albumin from AB Kabi, Stockholm The nitrogen content in this albumin is determined and protein concentration calculated (N-content taken to be 16%)

Further in a 2 week period, Ortho® Spinal Fluid Control, lot no 4P154 was analysed According to "assay data" supplied with the con trol the expected protein concentration was 0 80 g/l±0 12 g/l as determined on ACA® (Automatic Clinical Analyser, DuPont Company, Instrument Product Division, Wilmington, Delaware) with a turbidimetric method using trichloroacetic acid or 0 85 g/l±0 15 g/l determined with the turbidimetric method of Henry et al (1956) also using trichloroacetic acid

RESULTS

The results of reevaluating the method is seen from Tables I and II The comparison between the biuret and the Folin–Lowry method in Table I shows a slightly higher estimation of the protein concentration by the last mentioned method As has been stated by Cannon et al among others (1974) the photometric determination does not strictly follow Beer's Law, i e there is a slight deviation from the linear correlation between calculated protein concentrations in dilutions and the measured As a consequence, protein concentrations below the concentrations of the standard used (0 70 g/l) are overestimated, and concentra tions above are underestimated, in both cases it is less than 5% Specimens with concentra tions above 1 0 g/l are to be diluted and reanalysed So this observation has only little practical and quantitative importance

The measurements on the commercial CSF-control in Table II show good agreement with the values reported

Table III shows the results of protein determinations in CSF arranged in groups according to sex and age Males exhibit significantly higher values ($p=0\ 001$) than females No correlation existed between age and CSF protein concentration (Spearman rank correlation test)

No difference between the psychiatric and orthopaedic patients could be shown (by the Mann Whitney test)

Figs 1 and 2 display the CSF protein distribution among females and males, Fig 3 the distribution in the total material

For practical purposes there is no reason to

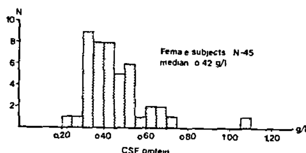


Fig 1 CSF protein in 45 female subjects

make distinctions between males and females as to the protein concentrations in CSF

The 0 05–0 95 fractile interval in the present total material is 0 29–0 88 g/l, which now is used as reference interval for this laboratory

DISCUSSION

Estimating the analytical bias (the accuracy) of the method in determining the protein concentration in heterogenous protein solutions as CSF (and serum) has certain shortcomings as the actual concentration of protein compo nents with varying chromogenicity in the actual sample is unknown

It has been claimed that the results are re latively unaffected by changes in the albumin/globulin ratio Results have also been reported to agree well with the total proteins determined by Kjeldahl analysis for normal CSF and diluted serum (Lous et al , 1956, Cannon et al 1974)

The results of re evaluating the methods as mentioned above exhibit an acceptably low analytical bias for quantitating total protein concentration in CSF

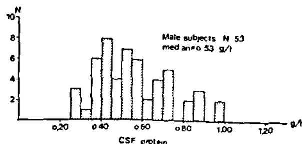


Fig 2 CSF protein in 53 male subjects

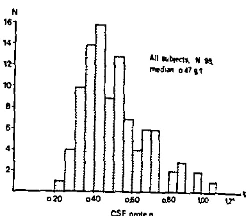


Fig 3 CSF protein in 98 subjects the total reference material

The results have shown a sex difference in CSF protein concentration, with higher values among men compared with women The difference is significant for the age groups up to 59 years, and for the total material This is in contrast to the findings of Jung et al (1973) and Tibbling et al (1977) In contrast to these authors we were unable to register any significant correlation between CSF protein concentration and age These authors did not attach any practical importance of their findings and neither did we find any reason to make distinctions between males and females for practical purposes as to the protein concentration in CSF

A 0 05–0 95 fractile interval of CSF concentration at 0 29–0 88 g/l with a median value of 0 47 g/l is one of the highest reference values found in the literature Tibbling et al (1977) found in 93 subjects a mean value of 0 43 g/l and a range of 0 27–0 80 g/l (estimated from figures) and Jung et al (1973) in 11 subjects, presented a normal range of 0 1[illegible]–0 41 g/l ($x \pm 2$ s)

The discrepancies in the CSF protein concentrations found in the literature (Jung et al 1973, Tibbling et al , 1977) may depend on the selection of the subjects included, the methods used for analyses and various standards adopted, e g diluted serum albumin, tyrosine or mixtures of tyrosine and albumin as mentioned by Tibbling et al (1977) Jung et al

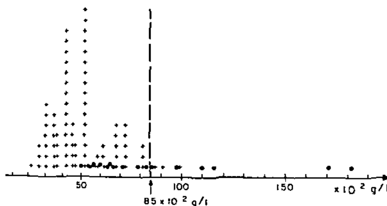
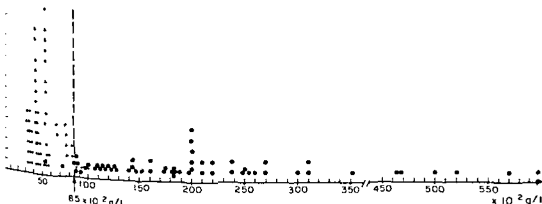


Fig 4 CSF protein in reference material (+) in relation to CSF protein in 16 patients with medium sized acoustic neuromas (●)

73) mention a possible geographical in ence on the CSF protein concentration
Our results confirmed the clinical impres- n that our previous reference values for F protein were too low

nical implications

has previously been reported by Thomsen 76) that among medium sized and large oustic neuromas the majority if not all had reased CSF protein, using an upper limit of g/l for the normal values This has also been reported by Pulec et al (1971), Fisch & Wegmuller (1974), Ojeman et al (1972), among others We therefore compared our previous CSF protein concentrations in operatively verified acoustic neuromas with the new reference material (Figs 4 and 5) The neuromas were 16 medium sized, in 10 female and 6 male patients, aged 25–67 years, and 61 large tumours, among 39 female and 22 male patients, aged 19–72 years Age and sexwise, they did not differ from the reference material Even though there is a statistically significant



F 5 CSF protein in reference material (+) in relation CSF protein in 61 patients with large acoustic neu mas (●)

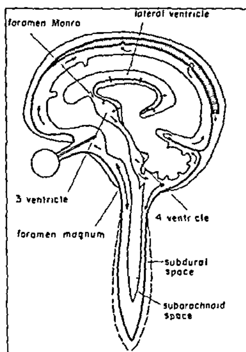


Fig 6 Schematic drawing of CSF flow

difference in total CSF protein between the reference material and patients with medium sized tumours ($p<0\ 001$), and between patients with medium sized and large tumours ($p<0\ 001$), using the Mann-Whitney test, there is so much overlapping among the patients with medium sized tumours and the reference group that only 37% exhibit abnormal CSF protein concentration, applying an upper limit of 0 85 g/l This indicates that the value of determining CSF protein in patients with medium sized tumours is most questionable Thus the difference between the reference group and the group of patients with medium sized tumours has only statistical but no clinical significance In contrast to this the difference between the reference group and the group of patients with large tumours has both statistical as well as clinical significance However, with a large tumour the patient will exhibit a bulk of other symptoms, and the diagnosis is easily made by other means (Thomsen et al , 1977) We therefore feel that the determination of CSF protein in the diagnosis of acoustic neuromas is of little if any value

There is an estimated daily production of 600–800 ml cerebrospinal fluid It is produced in the choroid plexus by ultrafiltration of plasma, and the circulation is indicated in Fig 6 through the ventricular system and from the 4th ventricle to the subarachnoid space through the foramen Monroi and foramina Luschkae The absorption or drainage of the cerebrospinal fluid is believed to take place in the large sinuses, as is also seen in Fig 6 Normal CSF proteins consist in 80% of proteins from plasma, the remaining 20% is produced locally in the subarachnoid space consisting of prealbumin, transferrin lysozyme β-trace, γ trace, and, in local infections or autoimmune disease, immunoglobulins The amount of CSF protein increases by increased blood–CSF barrier permeability and by blockage of parts of the liquor space (Schultze & Heremans, 1966)

Since the CSF protein elevation is mainly found in large acoustic neuromas it is most likely that the increase is due to a blockage of the circulation in the basal cisterns It is not clear, however, whether an acoustic neuroma also gives rise to a breakdown of the blood-brain–liquor barrier, or incurs a local production of proteins Barrier permeability is examined by determining CSF protein CSF albumin, or better CSF albumin/serum albumin ratio If the concentration of a protein in the spinal fluid increases due to local production the concentration must be compared with the barrier permeability This can be done by an index calculation as described by Bock (1977), Hansen et al (1977) and Tibbling et al (1977) If spinal fluid proteins are to be of diagnostic value in acoustic neuromas we feel that the determination should be supplemented by a more detailed examination of the protein content as described briefly above Such investigations are in progress

ZUSAMMENFASSUNG

Aus einem neuen Normalkollektiv von 53 Männern und 45 Frauen werden die Werte des Gesamteiweißgehalts (GEG) im Liquor Cerebrospinalis vorgelegt Seit 19

des Normalbereiches auf 0 88 g/l verlegt werden muß Dieses macht die Bedeutung des GEG in der Acusticusneurinomdiagnose außerst fraglich Unter den Tumoren mittlerer Große waren Fälle mit statistisch signifikant erhohten GEG Werten jedoch ohne klinische Signifikanz Die großen Tumoren zeigten zwar sowohl statistisch als auch klinisch signifikant erhohte GEG Werte aber diese Tumoren lassen sich leicht in anderer Weise diagnostizieren Eine differenzierte Bestimmung des GEG Wertes im Liquor Cerebrospinalis wird besprochen

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EVOKED POTENTIAL CORRELATES OF GENETIC PROGRESSIVE HEARING LOSS

Age-related Changes from the Ear to the Inferior Colliculus of C57BL/6 and CBA/J Mice

Kenneth R Henry[1] and Charles M Lepkowski[2]

(Received February 1, 1978)

Abstract Vol ...
were simul ...
the inferior ...
displays ge ... sensorineural *progressive hearing loss* was compared with the CBA/J *mouse At 50 days post* partum amplitudes of the summating potential (SP) and cochlear *microphonic* (CM) were lower in the C57BL/6 *genotype* and they decreased progress ...

... at the auditory nerve

The aging process in humans is often associated with a progressive sensorineural hearing loss, which has been related to both genetic and environmental factors (Schuknecht, 1974, Konigsmark & Gorlin, 1976, Hawkins, 1973) The pathology is typically described in reference to the inner ear, although auditory central nervous system (CNS) involvement has been reported in some cases of age-related hearing loss (Schuknecht, 1974, Bredberg, 1968) Although 10 to 15% of the adult population of the United States is significantly affected by hearing loss (Pagarella, Hanson, Rao & Ulvestadt, 1975) practical and ethical considerations make it difficult to experimentally evaluate genetic environmental, peripheral and CNS variables Adequate animal models would be useful for experimentally isolating these variables

Mikaelian, Warfield & Norris (1974) have described behavioral and anatomical evidence for genetic progressive hearing loss in the C57BL/6 mouse The present paper describes the application of computer averaging techniques to this animal model Measures of electrical activity from peripheral and CNS auditory structures were non traumatically obtained from the C57BL/6 mouse which support and extend the behavioral and anatomical evidence of Mikaelian et al (1974)

Electrocochleography utilizes electrodes placed at the promontory or external auditory meatus to non traumatically record inner ear electrical activity (Yoshie, Ohashi & Suzuki, 1967, Sohmer & Feinmesser, 1967, Aran & LeBert, 1968) The same principle (i.e. computer-averaging a large number of volume conducted evoked potentials) has been applied by Jewett (1970) to the auditory brain stem, and responses have been obtained which correspond to identified regions from the cochlear nucleus to the inferior colliculus (Jewett, 1970, Jewett & Romano, 1972 Buchwald & Huang, 1975) The present experiments simultaneously measured these peripheral and CNS auditory responses in genetically defective C57BL/6 (Mikaelian et al 1974) and normal hearing CBA/J (Finck & Berlin, 1965, Mikaelian et al., 1965, Shnerson 1971, Webster & Webster, 1977) mice from 50 to 200 days post partum, relating the results to experimental and clinical literature

METHOD

Mice of the highly inbred C57BL/6 and CBA/J strains were used for this study The subjects of the former strain were the 8th to 10th generation and the mice of the latter strain were the 2nd and 3rd generation of parental stock obtained from the Jackson Laboratory Only male mice were tested, to eliminate possible sex differences The animals were bred, reared and maintained in a quiet mouse colony (ambient noise, with the exception of that produced by the subject themselves, typically did not exceed 20 dB SPL at frequencies above 10 kHz) and were given no antibiotics, to reduce environmental influences on hearing loss From 8 to 11 mice of each genotype were tested at 50, 100 and 200 days of age Some mice were tested at a single age, while others were tested at 2 or 3 ages No effects of repeated testing were observed

The subjects were anesthetized with 60 mg/kg pentobarbital, i p , and maintained at a temperature of 37 5 (±0 1)°C, as measured with a microthermistor placed against the right axilla A modified version of the method of Jewett & Romano (1972) was used to obtain volume conducted responses from the right ear and from subsequent auditory brainstem regions Electrical activity was recorded between a scalp vertex stainless steel electrode (positive) and a steel bar pressed against the soft palate (negative) The vertex electrode was especially sensitive to activity generated in the brain stem while the mouth electrode emphasized responses from the ear (Plantz, Williston & Jewett, 1974)

Acoustic stimuli were generated by amplified 0 1 msec duration square waves, transduced by an Altec 801 tweeter, and delivered by a sound tube to the right ear of the subject At the level of the ear, the stimuli had their greatest energy at 2 kHz, with a 25 dB drop at 1 k and 10 kHz Clicks were presented at the rate of 20 per second at SPL's from 40 to 100 dB in 20 dB steps (all SPL's are re 0 0002 dynes/cm²) The bioelectrical signals were amplified by a factor of 10^6 with two adjacent cascaded Grass P15 preamplifiers with filter settings of 300 and 3 000 Hz They were recorded with a digital computer signal averager with an A to D conversion of 9 bits, at a temporal resolution of 10 to 30 μsec per address The evoked potentials were averaged from the responses to 512 clicks of alternating polarity At one SPL (80 dB), the responses to rarefaction and condensation clicks were analysed separately, but they were combined for all other measures All averaged responses were plotted, and latency values were directly obtained from the computer and transcribed A train of 2 kHz sine waves of 10 μV amplitude was used for system calibration Amplitudes of inner ear responses were measured from the pre stimulation electrical value to the peak of the evoked potential Brainstem evoked potential amplitudes were measured from the negative trough to the following positive peak Unless otherwise stated, t tests were used to evaluate these latency and amplitude measures

RESULTS

A typical volume conducted auditory response is seen in Fig 1 The summating potential (SP) is clearly visible, although alteration of the stimulus polarity has cancelled out the cochlear microphonic (CM) Previous experiments (K R H , in preparation) have shown that the SP and CM correspond to the summating potential and cochlear microphonic measured from the round window P_1 corresponds to the AP as measured from the round window and across the auditory nerve of the stimulated ear of the mouse The dual P_1 peaks correspond to high (P_{1a}) and low (P_{1b}) intensity threshold portions of the AP amplitude input–output function The brainstem components in the mouse have also been examined by local recording and lesion experiments (K R H , in preparation) and found to correspond with responses obtained from the cat (Jewett, 1970, Buchwald & Huang, 1975) The

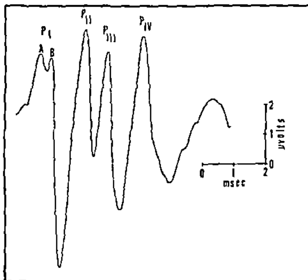


Fig 1 Volume-conducted auditory nerve (P_I) and brain stem (P_{II-V}) evoked potential from the laboratory mouse (see text for explanation)

responses in the mouse and cat are generated, primarily or exclusively, at or near the following structures P_{II}, the cochlear nucleus ipsilateral to the stimulated ear, P_{III}, the contralateral superior olivary complex, P_{IV}, the nucleus of the lateral lemniscus on both sides of the brain stem, and P_V, the contralateral inferior colliculus

At 50 days post partum, there were no genotypic differences in amplitudes or latencies of the P_{I-V} evoked potentials at any SPL Even though these neural values were *the same, sensory measures differed between* the two strains of mice The CM in response to a rarefaction click was 55 4% smaller in amplitude in the C57BL/6 than in the CBA/J mouse ($p<0$ 001), the condensation CM of the C57BL/6 was 48 3% ($p<0$ 02) and its SP was 43 5% ($p<0$ 005) of those seen in the CBA/J strain

By 100 days, the C57BL/6 mouse exhibited a *change in function of the auditory nerve at* high SPL's P_{Ib} was only seen at 100 dB in 2 of the 10 mice of this genotype, although it was recorded in 9 of the 10 100-day old CBA/J's (chi square $p<0$ 005) This situation remained unchanged at 200 days with 2 of the 8 C57BL/6 and 9 of the 10 CBA/J mice showing P_{Ib} in response to 100 dB clicks (chi ... $p<0$ 01)

The neural (P_{I-V}) response amplitudes of the C57BL/6 mouse progressively decrease with increasing age Since there were individual differences in the peak of the input–output functions (*i e, responses* maximal in some subjects at 100 dB while others peaked at 80 dB) amplitudes were compared at 60 dB Over the 150-day span of this experiment, the C57BL/6 P_I amplitude decreased by 72% ($p<0$ 001) P_{II} declined by 69% ($p<0$ 0001), P_{III}, by 71% ... P_{IV}, by 55% ($p<0$ 001), and P_V by ... ($p<0$ 10)

Although the CBA/J neural response amplitudes to this same 60 dB click ... variant from 50 to 100 days, decrement ... observed at 200 days of age By this age P_I amplitude decreases by 53% ($p<0$ 001) ... by 41% ($p<0$ 0001), P_{III} by 41% ($p<0$ 0...), P_{IV}, by 56% ($p<0$ 0001) and P_V by ... ($p<0$ 001)

We did not observe any significant age related latency changes in these geno... which corresponded to the amplitude ... ments at 60 dB described above The SP ... and P_{I-V} latencies in response to a 60 dB ... remained relatively constant over time

By 200 days, the sensory indices at ... had decreased in amplitude for both genot... *For the CBA/J mice, the rarefaction CM* ... decreased by 58% ($p<0$ 001), the ... tion CM, by 40% ($p<0$ 001) and the SP ... 54% ($p<0$ 005) These responses were ... small to be measured in all of the 200-day C57BL/6 mice Therefore, the sensory ... ment seems to be more pronounced than ... neural decrement in the older C57BL/6 whereas sensory and neural losses ... *be approximately* equal in the 200-day CBA/J genotype

In addition to these comparisons at a s... SPL, each genotype was compared for ... changes to clicks of increasing SPL It ... reasoned that rates of *amplitude* and la... changes over this intensity range could

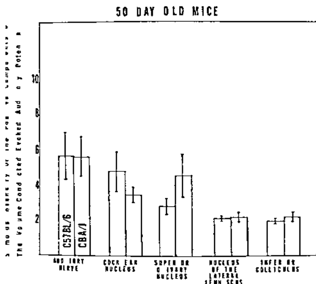


Fig 2 Relative amplitude changes as a function of stimulus intensity in C57BL/6 and CBA/J mouse genotypes at 50 days post partum (see text for explanation)

npared with similar indices which have en obtained from humans with various types hearing disorders These methods had other advantage Since they are indices of nge within each subject, genotypic com isons of these measures would mathemati ly cancel out such factors as bone density d growth rate which could affect con ctance of the evoked potentials to the ctrodes For the amplitude index, we di led the highest amplitude of a peak by the plitude of that peak at 40 dB For the latency measure, we subtracted the shortest latency of a peak from its latency at 40 dB Each measure was made for each peak, at every age, for each mouse This transformation also had the advantage of producing ratio and interval scales, which are most appropriate for powerful parametric statistical analyses

Figure 2 shows the mean relative amplitude changes (±1 S E) for neural evoked potentials in 50 day old mice The greatest dynamic change was seen at the auditory periphery, and this relative amplitude increase became less as the auditory CNS was ascended No genotypic differences were observed at this

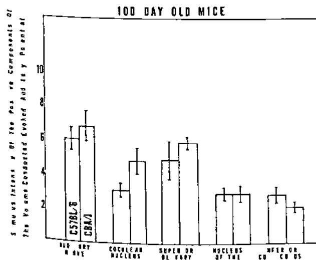


Fig 3 Same as Fig 2 at 100 days post partum

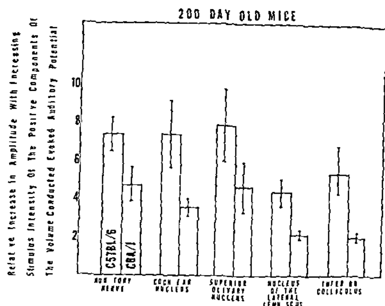


Fig 4 Same as Fig 2 at 200 days post partum

age By 100 days, this pattern begins to change (Fig 3) At this time, the C57BL/6 has a smaller dynamic range for the amplitude of EP from the cochlear nucleus ($p<0.01$)

By 200 days of age, however, pronounced genotypic differences were observed (Fig 4) in the rate of amplitude change to increasing click intensity The C57BL/6 developed a "recruitment" profile which is especially pronounced (in comparison with either 200 day-old CBA/J or with 50 day-old C57BL/6 mice) at higher brainstem levels Examination of the raw amplitudes of each subject indicated that these changed C57BL/6 amplitude ratios at 200 days were due to both an increase at high SPL's and a decrease at low SPL's and were not an artifact of the scaling technique The CBA/J showed no significant change in amplitude growth functions from 50 to 200 days of age

Even more pronounced CNS latency changes were observed as a function of age and genotype At 50 days of age (Fig 5) there were no genotypic latency differences and the latency decrease with increasing stimulus intensity was the same at all auditory levels A

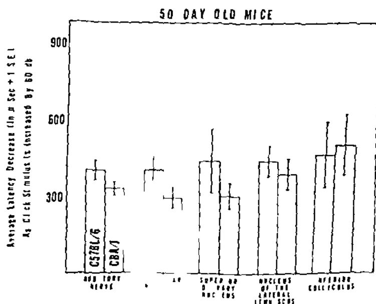


Fig 5 Relative latency changes as a function of stimulus intensity in C57BL/6 and CBA/J strains of mouse at 50 days post partum (see text for explanation)

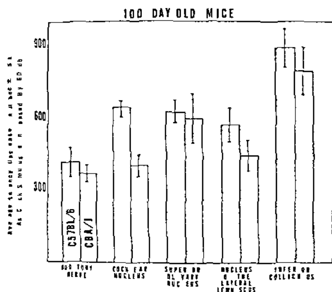


Fig 6 Same as Fig 5 at 100 days post partum

both genotypes aged, greater latency changes were observed in central than in peripheral structures, but there were still no genotypic differences at 100 days except in respect to activity from the cochlear nucleus ($p<0\ 001$, see Fig 6) By 200 days, however, there were major genotypic differences for CNS (but not peripheral) auditory regions At this older age, C57BL/6 mice have more pronounced latency decreases for $P_{II\ IV}$ than do CBA/J mice (Fig 7) This CNS effect was primarily due to an increase of latencies at 40 dB, with normal latencies being seen at high SPL's

DISCUSSION

The data of the present study support the behavioral and histological data of Mikaelian et al (1974), and their conclusion that the C57BL/6 mouse develops progressive hearing loss early in its life When sensory measurements (CM and SP) were made at 50 days of age, the values of the C57BL/6 were significantly lower than those of the CBA/J genotype This experiment also compliments the earlier behavioral audiograms with the inclusion of suprathreshold amplitude and latency functions, as well as measures of electro-

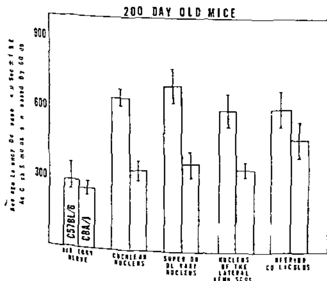


Fig 7 Same as Fig 5 at 200 days post partum

physiological changes which progress from the auditory periphery to higher brainstem levels

The electrophysiological deficits of the C57BL/6 mouse were first observed at puberty (50 days of age), slightly earlier than the age at which Mikaelian et al (1974) reported behavioral high frequency hearing loss, and considerably earlier than the age at which they first observed inner ear pathology in this subject The CM of the senile guinea pig was also found to be reduced in amplitude (Pestalozza et al , 1957) Crowley et al (1972*a*) reported similar CM decrements in the 24 month old rat To our knowledge, the SP has not yet been examined in the rodent with age related hearing loss

The auditory nerve action potential (AP, or P_1) of the C57BL/6 declined in amplitude within 50 days of the observed CM loss Crowley et al (1972*b*) also reported smaller AP's in the aged rat Bergholtz, Hooper and Mehta (1977), using transtympanic electrodes in the presbycusic human, noted a tendency for smaller AP s to be seen in subjects with increasing hearing loss

The waveform of the compound AP has been correlated with specific auditory dysfunctions (Aran, 1973, Eggermont, 1976, Yoshie, 1973) Bergholtz et al (1977) have associated certain AP waveforms with the presbycusic human, but feel that this presents only a crude estimate of cochlear damage The human AP obtained by transtympanic electrocochleography typically shows dual peaks, with the first mode being a short latency, high (H) intensity threshold response, while the second mode has a longer latency and a low (L) intensity threshold (Aran, 1973, Eggermont & Odenthal, 1974, Elberling & Salomon, 1976) P_{1a} and P_{1b} amplitude input–output curves from the mouse appear to correspond to the H- and L-curves of the human (Henry, in preparation) The observation that the aging C57BL/6 mouse is less likely to display P_{1b} at higher SPL's might indicate damage to those elements which contribute to the L curve Possible candidates include outer hair cells and the "tips" of the frequency-thresh[illegible] characteristic curves of single aud[illegible]ory [illegible] fibers (Eggermont & Odenthal 1974 Evans 1975)

It has recently been suggested that the common practice of combining rarefaction and condensation responses can distort the [illegible] waveform Coats & Martin (1977) noted that in the human with high frequency hearing loss the condensation and rarefaction AP's were quite different in appearance This was only found to be the case with one of the [illegible] mice, so it is unlikely that the results [illegible] artifacts of this procedure

The only dynamic brainstem changes in 100 day old C57BL/6 mouse were found in cochlear nucleus (Figs 3 and 6) As stimulus intensity was increased, the cochlear n[illegible] (CN) evoked responses showed a significant[illegible] smaller amplitude increase [illegible] decrease than did corresponding CBA/J [illegible]ures This may be related to the selective vulnerability of the CN (in relation to other auditory brainstem nuclei) to metabolic (Fisch, 1970, Dublin 1976) or to the maturity (11 to 15 days post partum) of [illegible] nucleus in the mouse (Mlonyeni 1967) congenitally sensorineural deaf [illegible] mouse deprived of sound from birth also [illegible] neural changes which are localized to the brainstem area (Webster & Webster 1977)

The 200-day-old C57BL/6 mouse show[illegible] "recruitment" of central (but not peripheral) auditory evoked potentials (EP's) relative to its 50-day old self, i e , the EP amplitude grows relatively larger with increasing stimulus intensity in the older C57BL/6 mouse The CBA/J mouse has no relative amplitude changes over this same age span This "recruiting profile" has been observed in the input–output function associated with recruitment of loudness (Yoshie 1968 Aran 1973) If the older C57BL/6 mouse displays loudness recruitment, these data would suggest that reduced input to the cochlear nucleus might be involved in the process

The CNS changes of the aging C57BL

mouse were not uniform at all neural levels The decline of the CN and superior olivary complex (SOC) EP amplitudes mimicked that of the AP (69–72%) while a smaller (49–55%) decrease was observed at higher neural levels This resembles the difference in P_I (AP) and P_V (upper brainstem) EP amplitude decrement which Sohmer & Pratt (1975) described in humans subsequent to noise exposure

Although the CBA/J mouse has been considered as having normal hearing (Finck & Berlin 1965 Mikaelian et al , 1965, Mikaelian, 1966 Birch et al , 1968, Sher, 1971, Webster & Webster, 1977), decrements were observed in all the electrophysiological measures by 200 days of age The relatively uniform pattern of this loss suggests one of two possibilities 1) CBA/J s develop a slight hearing loss which is sensory (or possibly conductive) in nature by 200 days of age (it may increase at later ages) and CNS amplitude changes merely reflect this loss 2) CBA/J mice have no hearing loss, and these absolute amplitude changes merely reflect impedance changes in an aging mouse This interpretation does not apply to C57BL/6 mice because they have changes which differ at various neural loci, and also show relative changes with increasing stimulus intensity where impedance values remain constant) The relative minor age related changes of the CBA/J are in contrast to the progressive changes of the C57BL/6 mouse, in which sensory deficits during adolescence were followed within 50 days by functional changes of the auditory nerve and cochlear nucleus, with higher auditory CNS functional alterations becoming manifest at 200 days post partum

ZUSAMMENFASSUNG

Elektrische Antworten des Hirnstamms wurden an Mäusen im Bereich zwischen Ohr und innerem Colliculus mittels äußerer Elektroden gleichzeitig gemessen Die Maus C57BL/6 die einen genetisch verursachten sensorineuralen zunehmenden Gehorverlust aufwies wurde mit de[illegible] Jagen war [illegible] ow e d e [illegible] C57BL/6 n [illegible] fortschreitendem Alter zunehmend ab Weitere 50 Tage spater wurden in den Antworten des Gehornervs und des cochlearen Kerns derselben Maus Veranderungen fest gestellt Im Alter von 200 Tagen wies die Maus C57BL/6 in den CNS Bereichen jedoch nicht am Gehornerv Anzeichen von Erholung auf

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SINGLE CELL LAYER MEMBRANE COVERING THE DEGENERATED COCHLEAR DUCT AFTER PERILYMPHATIC PERFUSION OF STREPTOMYCIN

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(Received February 10 1978)

stract Ultrastructural changes of the extrasensory [illegible]

…ts of the outer sulcus cells and finally involved other …thelial cells such as Claudius and the inner and outer …cus cells In the final stage except for the stria vas…laris and Reissner s membrane all epithelial cells which …ed the cochlear duct were replaced by a single-cell layer …embrane which originated medianly from the epithelial …lls of Reissner s membrane and laterally from the super…ial outer sulcus cells

…ecently, the function of epithelial cells lining …e cochlear duct have been discussed by …any investigators One can classify these …pithelial cells into organ of Corti, stria vas…ularis spiral prominence cells, outer sulcus …ells interdental cells, Reissner's membrane …tc and presumably each of these cell groups …as a certain functional role as well as func…ioning as a peri endolymph barrier

The purpose of this study was to investigate …lectronmicroscopically the fate of these cell …roups after perilymphatic perfusion of oto…oxic drugs Despite many reports about …hanges in the organ of Corti after administra…ion of ototoxic drugs, the pathologic changes …f extrasensory epithelium have scarcely been …mentioned In this study, we concentrated our …ttention on the pathological changes of the …xtrasensory epithelium We found that the …ining of the cochlear duct—except for stria …vascularis—is ultimately replaced by a single-cell membrane similar to that in reported temporal bone pathologies (Lindsay et al, 1960, Nager, 1952, Beal et al, 1967) The origin of this single cell layer membrane, not previously reported, became clear in our study

We have already described the changes in the nerve elements of the organ of Corti of the same animals as used in the present investigation (Terayama et al, 1977) We will report the details of the changes in stria vascularis separately

MATERIALS AND METHODS

Identical materials and methods as described in detail in our previous paper (Terayama et al, 1977) were employed for this study

In group A, we perfused 23 cochleae of 17 guinea pigs with 20% combined streptomycin solution (SM solution) through the scala tympani, and the animals were sacrificed after 3 to 137 days In group B, 8 cochleae of 4 animals were perfused with 2% SM solution and the animals were sacrificed after 31 to 76 days In group C, 8 cochleae of 7 animals were perfused with Ringer s solution and the animals were sacrificed after 7 to 137 days The cochleae were prepared for electron microscopy

In this paper, we obtained the results mainly from the 1st and 2nd turns of the cochlea



Fig 1 5 days after perfusion 20% SM Organ of Corti is collapsed due to distortion of supporting cells Hair cells are lost Inner sulcus cells and Claudius cells still appear normal The scale of the electron micrographs is indicated by a black bar with number, which represents μm

FINDINGS

(1) *Group A* (perfused with 20% SM solution)

In this group, the degenerative changes of the epithelial cells lining the cochlear duct—with the exception of Reissner's membrane—were outstanding The degree and extent of these pathologic changes increased with the time after the perfusion We classified them into early and later stage We found no rupture on Reissner's membrane On the lateral wall, all of three layers of the stria vascularis showed characteristic pathologic changes We will report the details of their changes in another paper

(a) *Changes in the early stage* (3 to 6 days after the perfusion) In the organ of Corti, hair cells had already disappeared The tunnel space was filled with the distorted and swollen supporting cells Claudius cells and inner sulcus cells appeared normal under the light microscope (Fig 1)

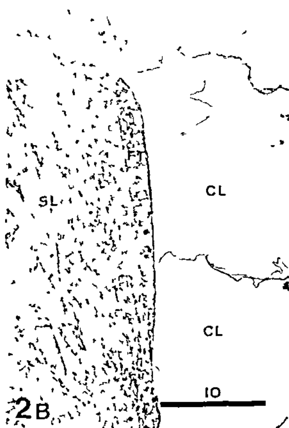
The spiral prominence epithelium appeared almost normal except that many filaments were conspicuous in their cytoplasm (Fig 2A)

The outer sulcus cells in 5 and 6-day specimens showed atrophy and degeneration and their cytoplasmic extensions (roots) had disappeared The space previously occupied by the roots became vacant except for some remaining debris In the cytoplasm of the cells underlying the superficial cells of outer and having direct contact with the degenerat roots, lysosome, vacuolization formation lamellated bodies, and lipofuscin granules in creased in number, and mitochondria de creased The superficial cells of the outer sulcus showed atrophic dark cytoplasm but with fewer degenerative signs than in the un derlying cells (Fig 2A)

Small flat cells were occasionally found be tween Claudius cells and the spiral ligament Claudius cells started their degenera already at this time These small flat cells con tained many vacuoles in the dark cytopl and seemed to be part of the outer sulcus ce (Fig 2B) In the later stage, they seemed become a lining on the basilar membrane spiral ligament, increasing their cytoplasm volume and number at the site of disappear Claudius cells

In the spiral limbus of the 4 and specimens, the cytoplasm of the interde cells was shrunken and contained many uoles and lipoid like materials of varying si (Fig 3A) In the 6-day specimens the ho zontal parts and bodies of the degenerated i terdental cells were on the verge of disappe ance In this stage, the single cell membra which seemed to derive from the limbus e thelium cells which were the epithelial cells Reissner's membrane, turned toward the u per surface of the spiral limbus and loca on it and/or epithelial cells of Reissner membrane began to extend toward the ves lar lip along the upper surface of the limb During this process they covered the u orifices of flask-shaped spaces left by the d appearance of the degenerated interde cells Much debris still remained in the v cated spaces (Fig 3B) Frequently, at t time, the inner sulcus cells started their d generation and the inner sulcus had no e thelial cells





...al prominence cell (B) 6 days after perfusion 20% ...l Flat cell (*FT*) between Claudius cells (*CL*) and spiral ...ment (*SL*) Arrows in the inset indicate the cor...ponding area

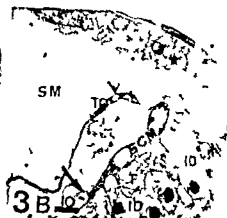
(b) *Changes in the later stage* (7 to 137 days ...er perfusion) On the lateral wall in the 9-day specimens, although their cytoplasm ...s condensed and their volume reduced, ...perficial outer sulcus cells still remained and ...vered the degenerated underlying cells ...hich were becoming vacant (Fig 4A) After ...e 9th day the underlying cells and roots of ...e outer sulcus cells completely disappeared

The spaces in the spiral ligament left by disappearance of the roots remained empty and after disappearance of the underlying cells their orifices to the endolymphatic space were closed by the cuboidal cells (Fig 4B) It was assumed that these cuboidal cells transformed from the superficial outer sulcus cells The cuboidal cells were contiguous with the flat single cell membrane which replaced Claudius' cells on the basilar membrane and extended to the collapsed organ of Corti (Fig 5)

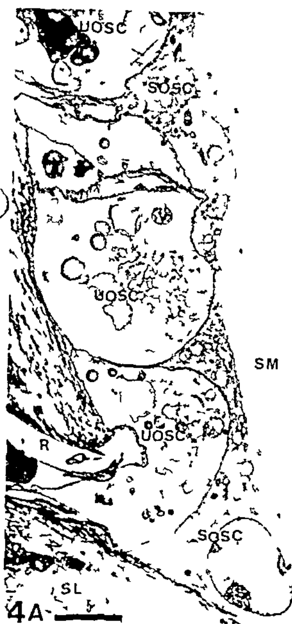
The spiral prominence epithelia were normal in shape but their cytoplasm were darker than normal due to increased filaments

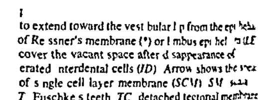
The tectorial membrane was detached from the degenerated interdental cells and was sometimes rolled up There were three types of detachment of tectorial membrane from the upper surface of the spiral limbus, 1) completely free from spiral limbus and floating in the scala media (Fig 6A), 2) barely





Fe 3 (A Sa o





I to extend toward the vest bular l p from the epi hela of Re ssner's membrane (*) or l mbus epi hel a (LE cover the vacant space after d sappearance of erated nterdental cells (ID) Arrow shows the area of s ngle cell layer membrane (SCM) SM scala T Fuschke s teeth TC detached tectonal membrane

F g 4 (A) 9 days after perfus on 0 SM Under cells (UOSC) of ou er sulcus cells lose normal cel ganelles and become vacant Superf c al cel (SC cover the degenerated underly ng cells R vacant after d sappearance of root cell SL sp ral ligament B days after perfus on 0 SM Cubo dal cell endolymphat c onf ces of vacant sp ces left by d sappe ance of roots (R) SM scala med a

R
R
SM
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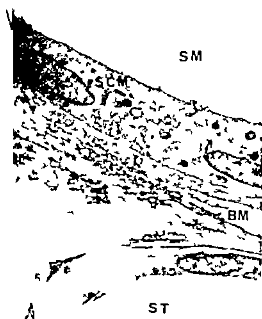


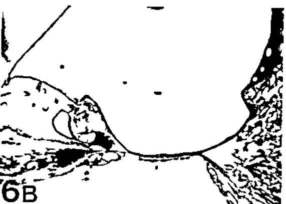
Fig 5 10 days after perfusion 20% SM Single-cell layer membrane (*SCM*) replace Claudius cells *SM* scala media *ST* scala tympani *SL* spiral ligament *BM* basilar membrane

the vestibular lip of spiral limbus (Fig 6B) 3) detached from spiral limbus and moved to the site of the degenerated organ of Corti (Fig 6C)

On the 8–9-day specimens the horizontal parts and the body of the interdental cell disappeared leaving flask shaped empty spaces in the spiral limbus The single cell layer membrane covered all upper orifices of flask shaped empty spaces (Fig 7A) and then enveloped the vestibular lip in 9 day specimens (Fig 7B) and with progress of time reached the inner sulcus cells (Fig 7C) If the rolled up tectorial membrane was connected with the spiral limbus the single cell layer membrane proliferated over the tectorial membrane

The cells of the single cell layer membrane were contiguous with the epithelial cells of Reissner's membrane and were morphologically quite similar to them They were cuboidal or flat in shape and had many vesicles well developed Golgi complex endoplasmic





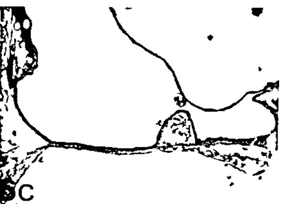
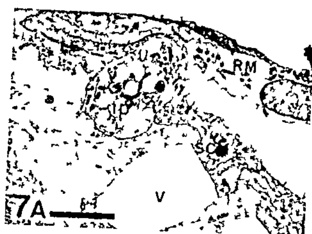
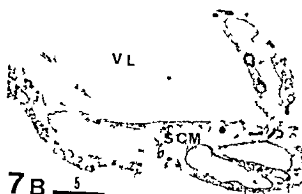


Fig 6 Fate of tectorial membrane (A) 10 days after perfusion 20% SM Completely free from the spiral

membrane







reticulum multivesicular body and s... mitochondria in their cytoplasms They ... many microvilli on their endolymph... s... face and had a basement membrane on ... side of spiral limbus (Fig 7) The cell ... possessed junctional complexes near th... dolymphatic surface and interdigitating ... cesses The morphological appearance ... single cell layer membrane from the la... wall of the cochlear duct (Fig 5) was ... same as the above described single-cell la... membrane from the spiral limbus

After the disappearance of the inner s... cells in the more advanced stage the ... cell layer membrane replaced them ... tended to the collapsed organ of Corti ... single cell layer membrane finally co... organ after merging with the single-cell la... membrane originating from the lateral side ... 12–17 day specimens (Fig 8) After the ... pearance of the supporting cells in the ... Corti macrophages fibroblasts myel... and unmyelinated nerve fibers and somet... rolled up tectorial membrane were fo... neath the single cell layer membrane (Fig 9...

In the 137 day specimen the degenera... process appeared to cease as fibrocytes ... macrophages were almost non existent in ... organ of Corti Except for the stria vascula... and Reissner s membrane the epithelial ce... normally lining the cochlear duct were ... lost After their disappearance the single-ce... layer membrane covered the spiral ligam... the basilar membrane and the spiral ... but did not replace the stria vascularis e... though their thickness had decreased (Fig ... In some specimens even in this stage ...

Fig 7 Single cell layer membrane (*SCM*) on limbus (A) 10 days after perfusion 20% SM Single layer membrane continues from the epithelial ce... Reissner s membrane and covered the vacant spaces by disappearance of interdental cells *RM* ... membrane (B) 10 days after perfusion 20% SM E... veloping the vestibular lip of spiral limbus (*SLP*) (C) ... days after perfusion 20% SM Reaching the inner ... cell (*ISC*) Arrow in the inset shows the corresponding area in same specimen



Fig. 8. [illegible] epithelial cells of Reissner's membrane at the site of Corti's organ. *D*: degenerated cells of organ of Corti. *HA*: Habenulla perforata. *BM*: Basilar membrane. *TM*: rolled up tectorial membrane covered by the single-cell layer membrane. Arrows show the spearhead of the single cell layer membranes.

distorted tectorial membrane covered by single-cell layer membrane was still recognized on the basilar membrane or on the spiral limbus.

(2) *Group B* (perfused with 2% SM solution)

In this group, the pathological changes in the epithelial cells lining the cochlear duct were restricted to the hair cells in the organ of Corti. Although not all the hair cells underwent degeneration, the defects of the reticular lamina produced by loss of outer hair cells were replaced by the hypertrophied Deiters' cells and outer pillar cells. We found no further involvement even in animals kept alive for long periods. Degeneration of the outer sulcus cells and interdental cells was not observed and then extension of the single-cell layer membrane was not observed at the lateral wall and the median border of the spiral limbus. As described previously, the number of nerve fibers was reduced (Terayama et al., 1977).

(3) *Group C* (perfused with Ringer's solution)

In this control group, most of the organ of Corti and other epithelial cells lining the cochlear duct showed no pathological changes, even after long survival following the perfusion. A few Corti organs lost their outer hair cells, which were replaced by Deiters' cells and outer pillar cells as in group B.

DISCUSSION

It has been confirmed from tracer studies (Ilberg, 1968*a*, *b*, 1970*a*, *b*, Duvall & Suther-

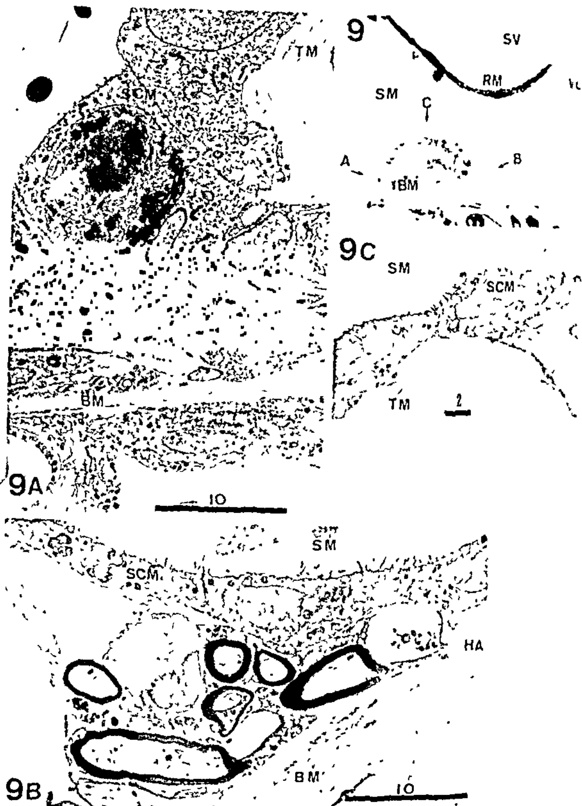
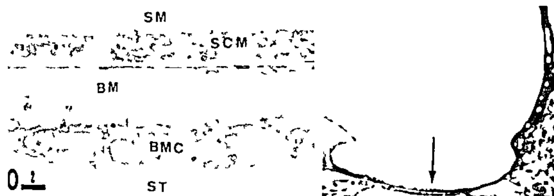


Fig 9 64 days after perfusion 20% SM Single-cell layer membrane at the area of the organ of Corti which degenerated out Arrows in the light microscopic picture indicate the corresponding area in electron microscopic pictures *RM* Reissner's membrane *BM* Basilar membrane *VL* Vestibular lip of spiral limbus *SM* Scala media *SV* Scala vestibuli (A) 9 days after perfusion 20% SM Lateral area of degenerated organ of Corti Under the single cell layer membrane, distorted tectorial membrane (*TM*), unmyelinated nerve fibers and fibrocytes are found Basilar membrane (B) 64 days after perfusion 20% [illegible] Median area of degenerated organ of Corti [illegible] and unmyelinated nerve fibers, and fibrocytes are [illegible] *BM* Basilar membrane *HA* Habenulla perforata (C [illegible] days after perfusion 20% SM Top of degenerated [illegible] of Corti Under the single-cell layer membrane [illegible] degenerated tectorial membranes (*TM*) are found



10 137 days after perfusion 20% SM Single cell er membrane (*SCM*) on the basilar membrane Arrow he light microscopic picture indicates the correspond ing area in same specimen *SM* Scala media *ST* Scala tympani *BM* Basilar membrane *BMC* Basilar membrane cell

id 1972 Hinojosa, 1972, Angelborg, 1974, hnke 1973, Gorgas & Jahnke, 1974) and servations on freeze fractured materials hnke 1974, Iurato et al , 1976) of the coch- a that the epithelial cells and their zonulae culudentes lining the endolymphatic duct nstitute the peri endolymph barrier These ithelial cells seem to have different prop ties regarding absorption of tracers in peri mph as pointed out by Duvall & Sutherland 970) and Hinojosa (1972)

In our study, when the specimens were erfused with the 20% SM solution, the de- neration started at different times in differ- nt epithelial cell groups hair cells of the or n of Corti were affected first, the interdental lls and roots of the outer sulcus cells second, nd finally other epithelial cells such as laudius' cells and the inner sulcus cells and ltimately they were replaced by the single ell layer membrane Only stria vascularis and eissner s membrane did not disappear and ere not replaced by the single cell layer embrane The time difference in degenera ion indicates the differences in absorption roperty and/or the difference in vulnerability f each cell group to SM

It was impressive that while the damage of pithelial cells lining the cochlear duct was ery severe and extensively spread when erfused with 20% SM, only hair cells were lamaged with 2% SM It seems that the pro gressive degeneration in the cochlear duct was only expected when the threshold concentra tion of SM was reached in the perilymph Ilberg et al (1974) concluded from their in- vestigation that signs of inner ear toxicity from aminoglycoside antibiotics appeared above their threshold value only in the inner ear fluid Their conclusion was concerned with the toxic effects on the sensory epithelial cells only, whereas our study also showed a similar result on the extrasensory epithelium

Although a single cell layer membrane cov ering the collapsed organ of Corti or "rolled up" tectorial membrane was repeatedly de scribed in reports on the cochleae of animals and humans of viral infection (Lindsay et al , 1960, Nager, 1952), acoustic trauma (Ward & Duvall, 1971) sudden deafness (Beal et al , 1967), Kanamycin administration (Benitez et al , 1962) and mechanical disruption of the or- gan of Corti (Duvall et al , 1969), they all failed to describe the origin of the single cell layer membrane We found in this study that it originated from the epithelial cells of Reiss ner s membrane and/or limbus epithelium cells medianly, and from the superficial outer sulcus cells laterally These two single cell layer membranes which originated from me dian and lateral sides merged on the site previ ously occupied by the organ of Corti before its degeneration

The repair reaction of epithelial cells of

Reissner's membrane after its traumatic rupture is well known (Laurence & Yantis, 1957, Duvall & Rhodes, 1967) We found a similar strong repair process in the single cell layer membrane There is a morphological resemblance between the epithelial cell layer of Reissner's membrane and the single-cell layer membrane, but its physiological significance is not clear Assumedly, the membrane develops in order to block communication between peri and endolymph

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Appreciation is extended to Dr Arndt J Duvall for offering his comments and suggestions in preparing this paper

ZUSAMMENFASSUNG

Ultrastrukturelle Veranderungen des extrasensorischen Epithels in der Scala media der Meerschweinchen Cochleae wurden 3 bis 137 Tage nach einer perilymphatischen Perfusion der Schnecke mit 20% Streptomycin beobachtet Die Degeneration begann im Cortischen Organ schritt zu den Interdentalzellen und den Wurzeln der außeren Sulcuszellen fort und umfaßte schließlich andere Epithelzellen wie Claudiuszellen und die inneren und äußeren Sulcuszellen Im Schlußstadium ausgeschlossen die Stria vascularis und die Reißnersche Membran wurden alle Epithelzellen die die Ductus Cochleae linnerten mit einer Einschicht Zell Membrane die zentral von den Epithelzellen der Reißnerschen Membran und seitlich von oberflachlichen außeren Sulcuszellen hervorgingen ersetzt

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A TEST BATTERY APPROACH TO THE INVESTIGATION OF SUSCEPTIBILITY TO TEMPORARY THRESHOLD SHIFT

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Abstract This study examined individual differences among various psychophysical measures that have been [illegible]ted previously as predictors of susceptibility to noise-induced temporary threshold shift (TTS) The test battery was administered to a group of five normal hearing young adults and consisted of the following proce[illegible] (1) critical intensity (CI) at 2 kHz (?) loudness [illegible] noise stimulus and two others using tonal fatiguers were utilized to assess susceptibility to TTS Results revealed that the test battery delineated tough from tender ears Furthermore the aural overload test was found to be a highly accurate predictor of TTS

Individual differences in susceptibility to noise trauma are well recognized For obvious reasons investigators have long attempted to develop a measure which might delineate those persons most susceptible to hearing loss from high noise levels Much of this research has focused on the temporary threshold shift (TTS) paradigm The rationale for using TTS was based on a hypothesis proffered by Temkin in 1933 (Ward 1973) Temkin suggested that the measurement of the temporary change in hearing sensitivity following a brief and moderately intense acoustic overstimulation provided a simple and valid estimate of eventual permanent threshold shift (PTS) incurred from more severe exposures to loud sound Despite considerable research efforts, however the relationship between TTS and PTS remains far from simple Known to complicate this relationship are the several variables which influence the amount of TTS an individual incurs For example the frequency, duration and intensity of both the exposure stimulus and the test stimulus effectively alter the subsequent shift in threshold sensitivity

Consequently, utilization of the TTS paradigm as a single *simple* test of general susceptibility has never been realized despite numerous attempts to do so Ward (1965 1968) for instance concluded that the best predictor of eventual PTS was the TTS that resulted from exposure to *that specific noise* to which the individual is routinely exposed in industry This very same feature of noise specificity associated with the TTS paradigm, on the other hand limits the clinical utility of TTS methods in hearing conservation programs To explain Ward s conclusion suggests that a single standard noise stimulus cannot be administered to all subjects Rather, the noise from the individual s specific work environment must be employed Hence, the use of the TTS paradigm as a test of susceptibility has recognized limitations

In search of alternatives and/or additions to the conventional TTS procedure, this study was designed to examine individual differences among various psychophysical measures that have been suggested previously as potential predictors of TTS and auditory fatigue More specifically, three tests, one of which incorporated a TTS strategy, were examined First, is the aural overload test, originally proposed by Lawrence and Blanchard (1954) as a

measure of susceptibility and later used in differential diagnosis (Lawrence & Yantis, 1956) Loudness discomfort level (LDL) was another measure used in this investigation Hood (1968) has suggested that LDL may be related to the amount of post-stimulatory fatigue an individual incurs The final measure, referred to as the critical intensity (CI) procedure, was conceived originally by Rüedi (1954) and later used by Ward (1965, 1968) In the traditional CI paradigm the subject is exposed to a fatiguing stimulus which is increased successively in level until a criterion amount of TTS is observed In the former investigations by Ruedi (1954) and Ward (1965, 1968) the only time intervening between successive exposures was that required for threshold determination That is, no recovery period was employed A modification of the CI concept was employed in this study Our procedure seeks to determine the CI at which maximum TTS shifts upward in frequency from the exposure frequency to one half octave above the exposure frequency, a feature which appears to distinguish adaptation processes from fatigue (Ward, 1973) The present CI procedure was modified further from the earlier techniques by allowing recovery periods between exposure levels

To assess susceptibility to TTS, three exposure stimulus/test stimulus combinations were used The first combination utilized broad band noise as the fatiguer and measured threshold at 4 kHz following exposure This combination was selected for two primary reasons First, there has been substantial research conducted in which a broad band noise/4 kHz combination was used (Lightfoot, 1955, Miller, 1958 Ward Glorig & Sklar, 1958, Humes, Schwartz & Bess in press) Secondly, a broad band fatiguer has been recommended as the most appropriate standard TTS index of susceptibility (Ward, 1965, 1968) In addition to the noise exposure condition, tonal overstimulation utilizing both high and low frequency fatiguing stimuli was employed

METHOD

Subjects

Five normal hearing adults (1 male and 4 females) ranging in age from 23 to 24 with mean age of 23 3 years comprised the s... of this study All subjects exhibited nor... tympanometric curves (normal amplitude... shape, middle ear pressure within ... mmH_2O), normal acoustic reflex thre... (less than 95 dB HTL at 0 5 through 4 kHz... the test ear, and pure tone air-conduc... thresholds of 5 dB HTL (ANSI 1969, for the frequencies 0 5, 1, 2 and 4 kHz

Apparatus

Pure tone air conduction screening of all ... jects was accomplished with a clinical ... agnostic audiometer (Grason Stadler M... 1701) Electroacoustic impedance meas... ments were obtained with an imped... bridge (American Electromedics E-83)

The fundamental and exploring tones ... aural overload test were supplied by two o... lators (General Radio Model 1309A and ... tronix Model SC502) and monitored ... both a frequency counter (Tektronix M... DC501) and an oscilloscope (Tektronix ... 5103 N) The output of the attenuator wa... to an electrodynamic earphone (Telepho... TDH-39, 10 ohm mounted in an MX-41... cushion)

In the assessment of LDL, the test s... was supplied by an oscillator (General R... Model 1309A), monitored by a ... counter (Tektronix Model DC 501) f... by a timer (Grason Stadler Model 1223... electronic switch (Grason Stadler ... 1287B) which maintained a pulse ... tion with an on time of 300 msec, an off ... of 700 msec and a rise/decay time of 10 ... The tone was then fed to an amplifier ... lett Packard Model 465A) and record... tenuator and finally to the same earpho... that used in the aural overload test

The systems used for the aural overloa... loudness discomfort procedures includ...

phones, were periodically checked for har-
nic distortion For the signals generated by
h oscillator, the levels of the 2nd, 3rd, and
harmonics at the earphone, were at least
dB below that of the fundamental tone In
ition linearity of the recording attenuator
s confirmed over the range of use (65 dB)
The clinical audiometer, employed in this
estigation for screening, CI, and determina-
n of TTS was calibrated in accordance with
ANSI 53 6 (1969) specifications In addi-
n the overall sound pressure level (SPL)
l the spectrum shape of the white noise
nulus used in the TTS studies were deter-
ned

Calibration checks were performed through
the investigation and upon completion of
study All testing was completed in double-
lled testing suites (IAC Series 1200) having
acoustic environment suitable for threshold
ermination (ANSI, 1960)

ocedure

e fatiguing nature of some portions of the
esent investigation required that the lis-
ing task be divided into three separate ex
rimental sessions During the initial session,
re tone air-conduction thresholds were ob-
ned in the test ear at octave intervals rang
from 0 5 to 4 kHz Electroacoustic impe
nce measurements were also made to estab-
h normalcy of middle ear function In ad
tion to the screening, one of the following
st categories was randomly selected for com
etion during the initial session with the re-
aining categories assigned to later sessions

(a) Loudness discomfort level measured in accordance with Morgan et al (1974) at frequencies 0 5, 1, 2 and 4 kHz
(b) TTS at 0 75 and 3 kHz recorded from 0 to 3 minutes post exposure following 3-minute exposures to 0 5 and 2 kHz pure tones at 100 dB SPL
(a) Aural overload thresholds as measured according to Fausti (1971) for the fundamental frequencies of 0,5, 1 and 2 kHz

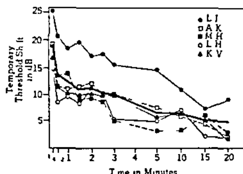


Fig 1 TTS at 4 kHz as a function of recovery time for the 5 subjects of this study Heavy line represents mean data for the group

(See Humes, 1977 for details of Fausti technique)
(*b*) TTS at 4 kHz recorded from 0 to 20 minutes post exposure following stimulation with thermal noise at 110 dB SPL for 5 minutes

3 CI procedure at 2 kHz
Sweep frequency threshold tracings from 2–4 kHz were obtained after exposure to a 2 kHz pure tone for one minute at 90, 95, 100, 105 and 110 dB SPL Ten minute rest intervals followed each exposure level

The procedural groupings were presented in a random fashion to avoid possible stimulus order effects Each session was followed by at least 24 hours of recovery after which pure tone thresholds at octave intervals from 0 5 through 4 kHz were redetermined If thresholds had not returned to resting level (±2 dB), another 24 hours intervened In addition subjects were instructed to avoid any noise exposure between test sessions

RESULTS

Temporary Threshold Shift (TTS)

The TTS obtained in the normal hearers at 4 kHz following exposure to 110 dB SPL broad band noise for five minutes is illustrated in Fig 1 The mean data are indicated by the heavy

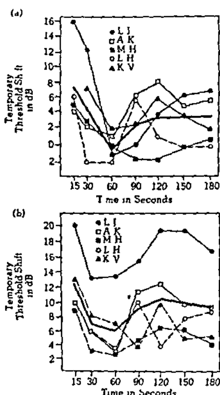


Fig 2 Individual variability in TTS as a function of recovery time Heavy line represents mean data for group *(a)* TTS at 0 75 kHz following exposure to 0 5 kHz pure tone at 100 dB SPL for 3 min *(b)* TTS at 3 kHz after stimulation with 2 kHz tone at 100 dB SPL for 3 min

solid line while individual data are marked according to the key of this figure Examination of the TTS at 2-minutes post exposure (TTS_2) was compared with predicted TTS derived from the formula developed by Ward, Glorig, and Sklar (1958) The predicted TTS_2 for the exposure conditions utilized in this investigation is approximately 12 dB As seen in Fig 1, subject L J demonstrated threshold shifts that far exceeded both predicted values and those of the other subjects Concurrently, subject M H demonstrated resistance to TTS As a group, however the mean results of the present study are in excellent agreement with those predicted according to the equation of Ward et al (1958)

Figure 2 illustrates the TTS recorded at 0 75 and 3 kHz following three minute 100 dB SPL exposures to 0 5- and 2 kHz pure tones, respectively The heavy line in Fig 2 *(a & b)* provides the mean data for the subjects C[...] sistent with previous research (Hirsh & W[...] 1952, Hirsh & Bilger, 1955) TTS re[...] were extremely variable across subjects A[...] in agreement with prior investigations n[...] appearance of a "bounce" in the short term [...] covery pattern (Hirsh & Ward 1952 [...] singer & Tillman, 1970) Using the mean [...] as an arbitrary limit, one subject (L J) co[...] sistently exceeded this limit and freq[...] yielded the highest value of TTS In add[...] as shown in the previous figure subject M [...] exhibited the greatest resistance to T[...] Thus, for these two subjects there appears [...] be a general condition of susceptibility [...] resistance to TTS, independent of the f[...] quency examined

In view of the consistency of the T[...] rankings observed thus far these data w[...] analyzed further via the Pearson *r* correla[...] index (Downey & Heath 1974) A high po[...] tive correlation was obtained between [...] noise induced TTS at 15 seconds p[...] exposure ($TTS_{0.25}$) for the 4 kHz test tone a[...] the tone induced $TTS_{0.25}$ recorded at b[...] 0 75 kHz ($r=0$ 86, $p<0$ 05) and 3 kHz ($r=0$[...] $p<0$ 05) For the TTS_2 values, a signif[...] correlation was obtained for the 4 kHz an[...] kHz test tones only ($r=0$ 80, $p<0$ 05)

Critical Intensity

The results obtained from the critical inten[...] procedure are illustrated in Fig 3 The [...] scissa represents the difference in TTS at [...]

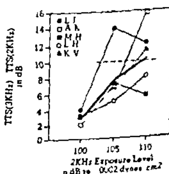


Fig 3 Mean (heavy line) and individual results obt[...] with the critical intensity (CI) procedure

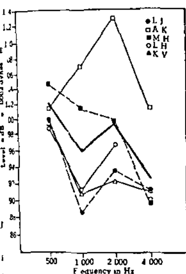


Fig 4 Mean (heavy line) and individual loudness discomfort levels (LDL) for the 5 subjects of this investigation

exposure frequency (2 kHz) and TTS at one half octave above this frequency (3 kHz) Data for the 90 and 95 dB exposures are not provided because a clear pattern failed to develop at these intensities The remaining exposure intensities, however, yielded an interpretable pattern At the 105 dB exposure intensity one subject (L J) exceeded an arbitrarily defined cut off value of 10 dB (same criterion as that used by Ward 1968) Following the 110-dB exposure subjects A K and L J demonstrated the greatest upward shift in TTS while subject M H displayed the least amount Overall, however, the CI levels from this study are compatible with those obtained by Ward (1968) using a similar procedure

Loudness Discomfort Level

Results of the LDL data are illustrated in Fig 4 With the exception of subject A K , individual data demonstrated a common pattern of frequency dependence The frequency dependence of the LDL estimates obtained in this study is comparable to that reported by Morgan et al (1974) although the present results are approximately 20 dB lower Subject L J is seen to display a generally lower mean LDL (average LDL for 0 5 through 4 kHz) while subject M H demonstrated a relatively higher mean discomfort level This ranking, therefore, is in reasonable agreement with the TTS ranking discussed above

Loudness discomfort levels, however, were found to be poorly correlated to TTS with coefficients varying between 0 26 and −0 17 It is important to note, however, that the subjects of the present study received no training with the experimental task prior to the determination of LDL On the other hand, it is desirable that a potential measure of susceptibility be as free from training restrictions as possible to facilitate broad and rapid application This apparent pitfall of the LDL procedure is discussed in greater detail below

Aural Overload

Thresholds obtained with the modified aural overload test are provided in Fig 5 Again with the exception of the 0 5 kHz fundamental tone, subjects L J and M H are at the extremes of the data continuum In addition, subject L J is clearly delineated from the group at frequencies 0 5 and 1 kHz When the group data were subjected to statistical anal

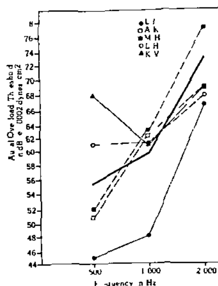


Fig 5 Mean (heavy line) and individual aural overload thresholds for fundamental frequencies 0 5 1 and 2 kHz

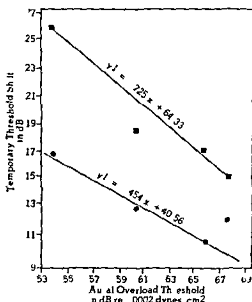


Fig 6 Prediction of $TTS_{0\text{-}3}$ (S E −1 05 dB) and TTS_2 (S E =1 92 dB) at 4 kHz from the mean aural overload threshold (0 5 1 and 2 kHz)

ysis aural overload thresholds were found to be significantly ($p<0\ 05$) correlated to TTS The aural harmonic threshold at 2 kHz was negatively correlated to $TTS_{0\ 25}$ ($r=-0\ 87$) and $TTS_{\cdot}$ ($r=-0\ 89$) for the 3 kHz test tone Furthermore the mean aural overload threshold (average overload threshold for 0 5, 1 and 2 kHz) was highly correlated to $TTS_{0\ 25}$ and $TTS_{\cdot}$ at 4 kHz The correlation between mean overload and $TTS_{0\cdot5}$ at 4 kHz was −0 98 while the correlation between mean overload and TTS_2 at 4 kHz was −0 84 Hence the lower the aural harmonic threshold the greater the TTS This finding is consistent with previous physiological hypotheses (Lawrence & Blanchard 1954 Drescher & Eldredge 1974)

In view of the relationship between mean aural overload and TTS at 4 kHz following exposure to thermal noise linear regression equations were calculated (Fig 6) The amount of $TTS_{0\cdot3}$ at 4 kHz following exposure to broad band noise of 110 dB SPL for 5 minutes can be predicted from the mean overload threshold by utilizing the following equation $Y=-0\ 725x+64\ 33$ where Y is equal to predicted $TTS_{0\ 25}$ in dB and x is equal to the mean aural overload threshold S ... TTS_2 (Y) can be predicted from mean overload (x) with the following formula $Y=-0\ 45[4]x+$ 40 56 The standard error of the regression lines was 1 05 dB for the prediction of TTS and 1 92 dB for the prediction of TTS

DISCUSSION

The statistically significant positive correlations among tone induced and noise induced TTS s at first appear to be in opposition to results obtained by Ward (1965 1968) W... found that those persons most susceptible to experimentally induced threshold shifts produced by high frequency stimuli were not necessarily the most susceptible to low frequency overstimulation That is a pattern of general susceptibility did not appear to exist in his subjects The correlation of $TTS_{0\ 25}$ found in this study for low and high frequency overstimulation however does not stand in opposition to Ward s conclusion Ward (1965 1968) was concerned primarily with measures of fatigue ($TTS_{\cdot}$ in this study) rather than adaptation ($TTS_{0\ 25}$) Adaptation has proven to be fairly constant as a function of frequency (Hood 1950) and should therefore be correlated across frequency Moreover failure to achieve a statistically significant correlation between TTS at 0 5 kHz and high frequency TTS_2 (3 and 4 kHz) is in accord with previous observations concerning auditory fatigue (Ward 1965 1968) It should be noted however that the adequacy with which low frequency TTS reflects underlying cochlear alterations has been questioned recently (Humes in press) The relative insensitivity of low frequency TTS may therefore be responsible for the lack of correlation between low and high frequency TTS observed previously by Ward and in the present study

Similar factors may govern the correlation between aural overload thresholds and T...

all that significant correlations observed in present study were restricted to high uency TTS Humes & Schwartz (1977) e commented on this frequency dependent elation Briefly they suggested that low-quency pure tone thresholds are less sensi to apical cochlear lesions than comparable rload thresholds The relative insensitivity w frequency pure tone thresholds to coch status has been noted previously by sev- investigators (Crowe, Guild & Polvogt, Bredberg 1968 Bohne, Eldredge, and 's 1973 Henderson, Hamernik and Sitler, Lipscomb, 1975a 1975b, 1975c, Corso, 6 Suga & Lindsay, 1976) Hence, a lack of relation between low frequency overload sholds and low frequency cochlear dam measured via pure tone thresholds may rely reflect the insensitivity of the latter nuli to alterations in the apical portion of cochlea

t has been suggested that a brief (3–5 min) monaural exposure to broad band noise, h as that used in this study, may represent singlemost effective predictor of general sceptibility to TTS aside from the specific se to which the individual is exposed ard 1965 1968) The high correlations ob ned between mean overload threshold and S induced by such a broad band noise, refore argues for the validity of aural over ad thresholds as predictors of overall sus ptibility to TTS Whether thresholds of erload prove to be accurate predictors of rmanent threshold shift awaits further inves gation Physiological data directed to this is e suggest that such a relation may prove true rescher & Eldredge 1974) As indicated in e introduction however, the TTS PTS re tionship is not a simple one Hence, the re tion of aural overload thresholds to PTS re uires d rect confirmation

The present investigation represents an itial step in developing a possible battery of sts used to delineate susceptible from non usceptible individuals The findings of this tudy suggest that both the aural overload test and the CI procedure possess sufficient sen sitivity to underlying individual differences in TTS Furthermore, as indicated by the present results, the effectiveness and applicability of the latter could be enhanced by restricting ex posure levels to 100 105, and 110 dB SPL and excluding the lower exposure intensities (90 and 95 dB SPL) This would also reduce the time of test administration considerably

It would appear, on the other hand, that loudness discomfort level is an inappropriate measure of susceptibility to TTS Not only were poor correlations of LDL to TTS es tablished in this study, but the determination of LDL itself is largely a function of instruc tions given to the subject the psychophysical method used, and the amount of subject training (Davis et al , 1946 Silverman 1947, Morgan, Wilson & Dirks 1974, Humes, 1976) It is the latter factor which possibly poses the most serious problem to the use of LDL as a measure of susceptibility The need for sub ject training requires more time than is often available in a screening program This is es pecially true if the test in question is one of several tests comprising a battery of measures

There are other measures of susceptibility that do not incorporate a TTS methodology and were not investigated here The threshold of octave masking (TOM) test (Clack & Bess 1969), for instance appears to be sensitive to individual differences in noise induced TTS (Humes 1977, Humes Schwartz & Bess in press) Another proposed index of susceptibil ity that has received relatively little attention is word discrimination score obtained in noise (Humes 1977 Humes, in press) Thus the present findings when considered with other recent research, suggest that the following procedures would comprise an ideal suscepti bility test battery (1) the aural overload test, (2) the TOM test (3) the CI procedure (ap propriately modified) and possibly (4) word discrimination scores obtained in noise The relative advantages and disadvantages of most of these tests has been discussed elsewhere (Humes 1977)

ZUSAMMENFASSUNG

Diese Arbeit [illegible] verschie[illegible] früher [illegible] rauschinduzierte zeitweise Schwellenverschiebung (TTS) vorgeschlagen wurden. Die Versuchsreihe bestand aus einer Gruppe von fünf normalen Hörern. Das Experiment bestand aus folgenden Stufen: 1) Kritische Intensität (CI) zu 2 kHz; 2) Lautheitsunbehagengrad (LDL) zu 0.5, 1, 2 und 4 kHz und 3) Ohrenüberbelastungsschwellen zu 0.5, 1 und 2 kHz. [illegible] die E[illegible] zu be[illegible] Beschallungsreiz verwandt und bei den zwei anderen Dauertöne. Die Ergebnisse zeigten, daß die Versuchreihe widerstandsfähige von empfindlichen Ohren unterschieden hat. Weiterhin zeigte sich, daß der Ohrenüberbelastungstest die zeitweise Schwellenverschiebung äußerst genau hervorsagen kann.

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COMBINED EFFECTS OF NOISE AND NEOMYCIN

Cochlear Changes in the Guinea Pig

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Abstract Cochlear damage resulting from the combination of neomycin with acoustic overstimulation was investigated in guinea pigs Four groups of animals received subcutaneous injections and exposure to broad band noise daily for 7 days as follows I Neomycin (200 mg/kg) followed by 10 hours of noise at 115 dB SPL II Saline followed by 115 dB noise III Neomycin followed by low intensity noise (45 dB as an acoustic control) or IV Saline followed by 45 dB noise After a 30 day stabilization period each ear was examined electrophysiologically and histologically Measures of cochlear integrity included AC cochlear potentials from 100 Hz through 20 kHz as well as outer hair cell (OHC) counts A marked interaction leading to augmentation of damage was found when neomycin was combined with 115 dB noise (Group I) Losses in cochlear sensitivity averaged across all frequencies amounted to 62 dB in Group I whereas the averaged losses for Groups II and III were only 16 dB and 17 dB respectively Loss of OHC's was close to 100% in Group I while OHC losses were only 17% in Group II and 26% in Group III

The ototoxic effects of the aminoglycoside antibiotics include damage to hair cells of the organ of Corti as well as changes in electrophysiological measures of cochlear function (Hawkins, 1959, Falk, 1972, Brummett, 1973, Ylikoski 1974) In general, damage occurs first in outer hair cells at the base of the cochlea With continued administration of the drug, outer hair cells toward the apex as well as inner hair cells may become involved (Ward & Fernandez, 1961, Benitez Schuknecht & Brandenburg, 1962, Reddy & Igarashi, 1962, Hawkins & Engstrom 1964)

Damage as a result of severe acoustic exposure is similar in many respects to that produced by the aminoglycoside an[illegible] whether the exposure consists of a pur[illegible] or a narrow band of noise Again outer ha[illegible] cells appear more vulnerable than inner ha[illegible] cells (Lurie, Davis & Hawkins 1944 Beag[illegible] 1965, Lim & Melnick 1971) However [illegible] extent the location of damage within the coch[illegible] lea is determined by the frequency spe[illegible] of the acoustic exposure (Stockwell Ades [illegible] Engstrom 1969, Fried Dudek & Bohne 19[illegible] Spoendlin 1976)

Because of the widespread use of the am[illegible] glycoside antibiotics and the presence of hi[illegible] levels of in hospital and environmental [illegible] there are significant possibilities for inter[illegible] tion between these two agents To date how[illegible] ever, there has been relatively little exper[illegible] mental investigation of such possibi[illegible] (Dayal Kosharian & Mitchell 1971 Jauha[illegible] nen Kohonen & Jauhiainen 1972 Dayal [illegible] Barek, 1975, Hawkins Marques & Cla[illegible] 1975, Marques Clark & Hawkins 19[illegible] Furthermore, the results from these investiga[illegible] tions are not in complete agreement with ea[illegible] other

The aminoglycoside antibiotic kanam[illegible] has received the greatest amount of atten[illegible] in regard to the possibility of an interac[illegible] with noise Histological examination of [illegible] cochlea in guinea pigs exposed to bo[illegible] mycin and noise, was first reported to [illegible] a potentiation of effect by Dayal et al [illegible] This work involved low level noise ([illegible]

Table I

Group	Acoustic exposure level*	Drug treatment
I Combination	115 dB	Neomycin sulfate (200 mg/kg)
II Noise alone	115 dB	Normal saline
III Neomycin alone	45 dB	Neomycin sulfate (200 mg/kg)
IV Procedure control	45 dB	Normal saline

*All sound pressure levels (SPL s) in this report are referred to 0 0002 μbar

and low doses of kanamycin (15–50 mg/kg) Potentiation of cochlear damage was also reported in work by Dayal & Barek (1975) using higher doses of kanamycin (100 mg/kg) and noise levels of 90 dB The duration of treatment in these studies was on the order of 3-5 weeks

More recent work has not confirmed the findings described above Hawkins et al (1975) and Marques et al (1975) have reported extremely variable findings after treating guinea pigs with various combinations of kanamycin and noise In these investigations both electrophysiological and histological measures were used, interaction between noise and drug was seen only in certain animals and only with noise exposures of at least 100 dB These investigations involved treatment durations of 7 days

The only other aminoglycoside antibiotic which has been studied in combination with noise is neomycin Augmentation of damage by combination of noise with neomycin was reported by Jauhiainen et al (1972), following daily exposure of guinea pigs to 115 dB of octave band noise combined with daily injections of neomycin (200 mg/kg) The duration of treatment was 7 days Both electrophysiological and histological measures were reported to demonstrate the augmentation effect.

The above results with noise and neomycin have not been replicated prior to the present report Because of the conflicting evidence concerning the possible interaction between the aminoglycoside antibiotics and noise, the present investigation was designed to determine whether in fact the observations concerning neomycin and noise could be confirmed In addition, although the study by Jauhiainen et al appears to have been carefully done, their electrophysiological measures did not extend to frequencies higher than 4 kHz In view of the well known tendency for ototoxic drugs to preferentially damage the basal portion of the cochlea the present investigation extended the observations to include frequencies up to 20 kHz

MATERIALS AND METHODS

Thirty-two healthy pigmented guinea pigs weighing between 200–400 g were selected for this study The animals were randomly assigned to one of four groups of 8 to receive the treatments as specified in Table I

The acoustic exposure was a broad band of white noise (Fig 1) The 115 dB exposure level was chosen to be consistent with the earlier work of Jauhiainen et al (1972) The low level exposure (45 dB) was added to provide an innocuous sound environment as an acoustic control

Exposure to noise was maintained for 10 hours/day over 7 consecutive days for a total exposure time of 70 hours During the sound exposure the animals were confined in groups of 16 within a 0 64 cm wire mesh cage (137 cm × 46 cm × 30 cm) suspended in a double walled, sound shielded room Water was available at all times Following each day's exposure the animals were returned to their home cages where food was also available

Noise was produced by a Lansing speaker (no 2483) driven by a random noise generator (General Radio no 1382) in conjunction with a filter (Bruel and Kjær no 2112) and a Dynaco 60 Watt power amplifier The speaker was suspended 153 cm above the cage floor and was fitted with an exponential ho se open end terminated 76 cm abov a floor

Sound pressure levels were measured with a sound level meter (Brüel and Kjær no 2203) on the linear setting Measurements were made at 2 inch intervals over the entire cage floor at heights of 1, 3, and 5 inches (624 points) The sound pressure level averaged over these points did not vary from 115 dB by more than ±2 dB

Injections of neomycin (200 mg/kg) or of normal saline (drug vehicle in equivalent volume per body weight) were given subcutaneously immediately before each sound exposure period All injections were given using a blind procedure

Following the 7-day period of injections and acoustic exposure, an interval of 30–40 days was allowed for damage effects to stabilize During this time the animals were kept in their home cages with no further exposure to drugs or to noise other than the ambient noise level within the laboratory

At the end of the stabilization period each animal was anesthetized by an intraperitoneal injection of allobarbitol (DIAL, 60 mg/kg) with urethane (240 mg/kg) A tracheal cannula was inserted and the animals were maintained on artificial respiration Body temperature was maintained at 38°±2°C

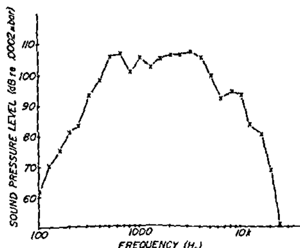


Fig 1 ⅓ octave sound pressure spectrum of the broad band of white noise used as acoustic exposure measured at 115 dB SPL Data points are at the center frequencies of each ⅓ octave

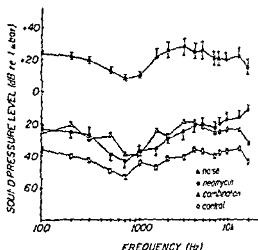


Fig 2 Averaged 1 μV isopotential functions of the AC cochlear potential obtained from all ears of four groups of animals Vertical range bars represent +1 standard error of the mean Major losses are seen in the group which received loud noise in *combination* with neomycin (top of figure) Exposure to *noise alone* or *neomycin alone* resulted in much less damage *Control* group is seen at bottom of figure

Both ears of each animal were prepared for the recording of AC cochlear potentials from the round window membrane using a silver electrode with a round ball tip For presentation of the acoustic test stimuli a specially designed sound cannula containing a calibrated probe tube microphone (Vernon & Meikle, 1974) was sealed into the external auditory meatus The sound cannula was connected to a loudspeaker (General Radio no 555) by 25 cm of soft rubber tubing and 35 cm of rigid walled tubing

Acoustic test stimuli consisted of pure tones at 16 frequencies from 100 Hz through 20 kHz (see Fig 2) stimulus level was measured at each frequency in each ear The AC cochlear potentials elicited by these stimuli were amplified 1 000 times and then measured using a narrow band wave analyzer (General Radio no 1900-A) The electrophysiological measures included (1) a measure of the amount of sound required to produce 1 μV of AC cochlear potential (the 1 μV isopotential function) at the above frequencies and (2) a measure of the magnitude of the AC cochlear potential

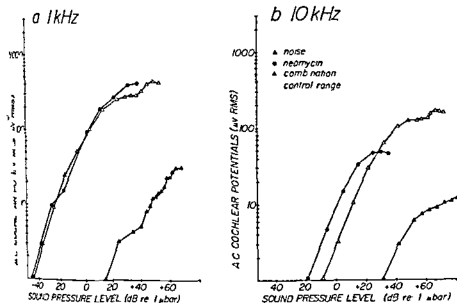


Fig 3 Intensity functions of the AC cochlear potential (a) 1 kHz and (b) at 10 kHz Each curve represents the median ear from one of the three experimental groups Stippled area shows the complete range of control values

resulting from a sound stimulus of increasing intensity (intensity functions) at 1 kHz and 10 kHz Electrophysiological measures are reported in terms of RMS voltage Tests were periodically made for radiation artifact (Vernon & Meikle 1974)

Immediately following the last electrophysiological measure, the cochleas were perfused with Dalton's solution containing 1% OsO_4 as a fixative The ears were further prepared for microscopic examination using the surface preparation technique (Engstrom, Ades & Andersson, 1966) The organ of Corti was dissected free from the osseous spiral lamina and sections were mounted in glycerine Counts were made of missing outer hair cells (OHC's) in segments of 80 OHC lengths at the base of the cochlea, turn 2, turn 3, turn 4 and at the apex Inner hair cells were not counted but any damage to these cells was and recorded

RESULTS

A marked interaction between noise and neomycin was confirmed by both the electrophysiological and histological measures The combination of these agents was far more traumatic to the ear than either agent given alone

Electrophysiological measures

The mean 1 μV isopotential curve for each of the treatment groups is shown in Fig 2 In agreement with earlier work (Jauhiainen et al , 1972), damage resulting from the combination of noise with neomycin was extreme The average dB loss for the Combination group (averaged across all frequencies, relative to Control group levels) amounted to 62 dB, while for the groups receiving noise alone and neomycin alone the average losses were only 16 dB and 17 dB respectively (Fig 2)

A similar effect was demonstrated by the intensity functions at 1 kHz and 10 kHz (Fig 3) The groups receiving noise alone or neomycin alone were close to or within the range of the control values, while the Combination group was severely depressed relative to the Controls Intensity function curves for the latter group were, in general, far to the right on the abscissa, indicating dramatic losses

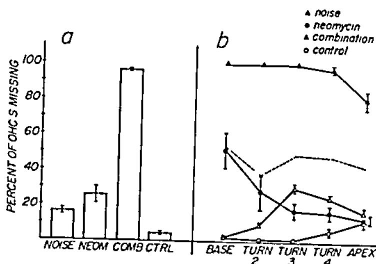


Fig 4 Results of outer hair cell counts expressed as a percentage of the total number that would exist in an equivalent segment of undamaged organ of Corti Counts averaged over all areas of the cochlea are shown for each group of animals in (*a*) these counts are represented as a function of place in the cochlea in (*b*). Vertical bars represent ±1 S E M The dashed line represents the sum of effects below it at each point

in sensitivity, and substantially depressed in height, indicating losses in the output capability of the ear Table II, which shows the maximum output for each group as averaged values, indicates the augmentation effect clearly

Noise alone and neomycin alone exerted approximately equivalent effects over most of the frequency range (Figs 2, 3*a*) At frequencies above 5 kHz, however, neomycin alone led to slightly more damage than did noise alone (Figs 2, 3*b*) The same comparisons are evident in Table II

In addition, it is interesting to note that variability within the two groups receiving neomycin was considerably greater than in the Noise alone or the Control group In these latter two groups variability was extremely small

Table II *Maximum output of the AC cochlear potential at 1 kHz and 10 kHz Values are the means of all ears within each treatment group*

Group	1 kHz	10 dHz
Noise alone	404 μV	185 μV
Neomycin alone	377 μV	51 μV
Combination	24 μV	9 μV
Control	1 264 μV	318 μV

Histological measures

Total OHC losses for each group expressed as percentage values relative to the total number of OHC s that would exist in an equivalent segment of undamaged organ of Corti are shown in Fig 4*a* These OHC losses are depicted as a function of cochlear turn location in Fig 4*b* We can see the dramatic exacerbation of damage effects in both Fig 4*a* and Fig 4*b* Not represented in the figure are the inner hair cell losses which were very small in the single agent groups but nearly 100% in the Combination group

Within every cochlear turn the number of missing OHC s in the Combination group exceeded the number missing in the Noise-alone, Neomycin alone, and Control groups combined This comparison is indicated by the dashed line (Fig 4*b*) which represents the arithmetic sum of the damage effects below it at each point Again variability was greater within the Combination and Neomycin alone groups than within the Control or Noise-alone groups

DISCUSSION

There appears to be an interaction between noise and neomycin as shown by the Combination group That is, the magnitude of the

osses when noise was combined with neo mycin was far greater than the simple addition of losses due to noise alone and neomycin alone

In general the present results confirm the observations of Jauhiainen et al (1972) Some differences however, do exist While this earlier report showed noise to have produced consistently more damage than neomycin at frequencies up to 4 kHz in the present study these two agents exerted approximately equivalent effects over most of the frequency range At frequencies above 5 kHz we found damage due to the neomycin alone was greater than that due to noise alone This may be seen in the isopotential functions as well as by comparison of intensity functions at 1 kHz and at 10 kHz In the earlier study, electrophysiological observations did not extend above 4 kHz which prevents a direct comparison of data above that frequency.

There was a good correspondence between the electrophysiological and histological results of the various treatments The nearly total destruction of OHC's in the Combination group was consistent with the dramatic depression of the isopotential and intensity functions in this group Moderate electrophysiological depression in the Noise alone and Neomycin alone groups was associated with relatively modest OHC losses in most areas of the cochlea Neomycin alone, however, led to a more dramatic destruction of OHC's in the basal portions of the cochlea This finding is consistent with the greater depression of cochlear potentials at high frequencies due to this agent Additionally, neomycin treatment appears to have led to more variable results than did noise treatment as seen both electrophysiologically and histologically

The fact that neomycin and noise, when given alone, tended to concentrate their effects in different parts of the cochlea (neomycin in the base, noise in Turn 3) might have led to the suggestion that the interaction occurring in the Combination group resulted simply from the compounding of drug induced damage in the basal turn with noise induced damage in Turn 3 This cannot be the case, however, for Fig 3 clearly shows that damage in both Turn 3 and the basal turn, in the Combination group, far exceeded that due to either agent alone in those same locations

The mechanisms of the interaction between noise and neomycin remain to be elucidated Neomycin has been shown to interfere with the turnover of phosphinositide lipids of the organ of Corti and stria vascularis (Schacht, 1976) These lipids are thought to be involved in the control of membrane permeability It was suggested in that report that exposure to neomycin may result in conformational changes in cell membrane structure leading to disruptions in cell organization and function Such disruption might render a sensory cell more susceptible to mechanical damage by acoustic exposure

Alternatively, noise exposure may lead to alterations in the pharmacokinetics of the drug For example, noise induced changes in general physiological state may affect the distribution, metabolism, or excretion of the drug in animals exposed to high levels of noise In this case it should be possible to demonstrate higher drug levels in the plasma as well as in perilymph of the noise exposed animals Another possibility is that noise exposure exerts an effect on the pharmacokinetics of the drug that is localized to the ear (Marques, Clark & Hawkins, 1975) In this case the noise exposed animals might show elevated drug levels in perilymph, but not in the plasma Further investigation is needed to determine whether any of the above mechanisms participates in the interaction between noise and neomycin reported here

ACKNOWLEDGEMENTS

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ZUSAMMENFASSUNG

Schädigung der Cochlea durch Neomycin kombiniert mit akustischer Überstimulierung wurde an Meerschweinchen untersucht Vier Tiergruppen erhielten 7 Tage lang tägliche Injektionen und wurden Breitbandlärm ausgesetzt wie folgt I Neomycin (200 mg/kg) mit 10 Stunden Lärm von 115 dB Lautstärke II physiologische Kochsalzlösung mit 115 dB Lärm III Neomycin mit schwachem Lärm (45 dB als akustische Kontrolle) oder IV physiologische Kochsalzlösung mit 45 dB Lärm Nach einer 30tägigen Stabilisierungsperiode wurde jedes Ohr elektrophysiologisch durch Messung der Cochlea Wechselstrompotentiale zwischen 100 Hz und 20 kHz und histologisch durch Zählen der äußeren Haarzellen untersucht Es wurde eine deutliche Wechselwirkung zwischen Neomycin und 115 dB Lärm (Gruppe I) gefunden die zu einer verstärkten Schädigung führte Der Empfindlichkeitsverlust der Cochlea betrug im Durchschnitt von allen Frequenzen 62 dB Der durchschnittliche Verlust in den Gruppen II und III war nur 16 dB und 17 dB Der Verlust an äußeren Haarzellen betrug fast 100% in Gruppe I während nur 17% in Gruppe II und 26% in Gruppe III verlorengingen

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PATULOUS EUSTACHIAN TUBE

Diagnostic Evaluation by Sonotubometry

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Abstract Twenty five patients (31 ears) with pathologically patent Eustachian tubes are presented and the symptoms are analysed All the ears were examined by sonotubometry and two different opening patterns could be separated for the patulous tube The variations in impedance of the ear during respiration were recorded from nearly all ears and three ears with eardrum perforation were also investigated by means of the airpressure equalization method Only sonotubometry brought out clearly the patulous Eustachian tube in all the ears examined It is thus a useful addition for diagnosing the abnormal patency of the Eustachian tube and for following up the results of its treatment then the explanation of the cure does not remain open to conjecture

The middle ear is normally aerated when the Eustachien tube is opened by the combined action of levator and tensor veli palatini muscles during swallowing The most common malfunction of the auditory tube is the inability of the tube to open and less commonly, the failure of the tube to close However the latter, a patulous Eustachian tube is probably not always recognized and may be easily under diagnosed and mismanaged

Several authors (Perlman 1939, Moore & Miller, 1951, Suehs, 1960 Miller, 1961, Flisberg & Ingelstedt, 1970, Munker, 1977) have reviewed in detail the symptoms and etiology of the patulous tube If the physician is familiar with this disorder and the signs and symptoms present are distinct the diagnosis will be easily established The diagnosis can even be discovered by the characteristic history of the patient alone The feeling of the occlusion or pressure, the hearing of one's own voice and respiration sounding louder than usual and the sensation of the head as in an empty barrel or in a tunnel (echoing) are the usual complaints about the ear concerned The symptoms may be relieved during an upper respiratory tract infection, by lying down, by bending the head forward between the knees, or by a sharp inspiratory effort through the nose with nose and mouth held closed (sniffing) or performing the reverse Valsalva manoeuvre Moreover, on examination the tympanic membrane can be observed to move in and out synchronously with respiration

The symptoms may also be intermittent or recurrent and findings may be normal on examination and the diagnosis may remain in doubt On the other hand, the tube may be patulous without giving rise to any complaints However, as a rule an abnormally patent Eustachian tube causes the patient annoyance and anxiety, sometimes even more than the periodically obstructed tube

In the present paper the results of a new method of examination—sonotubometry (Virtanen, 1977 1978)—are reported as applied for the diagnosis of the patulous Eustachian tube

MATERIAL AND METHOD

The material is composed of 25 patients (31 ears) with some of the following present or anamnestic symptoms and signs of the patulous Eustachian tube feeling of occlusion or fullness in the ear, hearing of one's own voic (autophony) and/or breathing directly in affected ear, relief by lying or putting

down and/or the moving of the eardrum synchronously with respiration (examined by Siegle's otoscope) The ages ranged from 27 to 83 years with a mean age of 40, in 15 women and 10 men 21 patients (27 ears) had intact eardrums and in 13 cases (16 ears) out of these there was a negative history of ear disease Three patients (3/25) had a dry tympanic membrane perforation, and no signs of infection were evident in the ears and one (1/25) subject had an atrophic eardrum All subjects were normal on examination of the nasal cavity, pharynx and rhinopahrynx showing no definite abnormality and the orifice of the Eustachian tube appeared normal on both sides

The Eustachian tube function was determined by the sonotubometry previously described in detail (Virtanen, 1977, 1978) The main principle of this method is to introduce the pure tone of 6, 7 or 8 kHz through the nasal olive tip into the nostril The test sound passing through the Eustachain tube during tubal opening is picked up by the microphone embedded in the circumaural ear defender and fitted with a probe of suitable size in the external ear canal The amplified output of the microphone is fed through the narrow band-pass filter in order to suppress background noise and is recorded by a level recorder in dB SPL re 20 μPa

For the sake of comparison the three ears with tympanic membrane perforation were also investigated by means of the air pressure equalization procedure according to Flisberg et al (1963) and Holmquist (1969) with some modifications This ventilatory function of the Eustachian tube was studied by means of an electroacoustic impedance bridge (Teledyne Model TA 2C) the manometer section of which was used A negative pressure level of -200 mmH_2O was produced by means of the air pressure pump of the impedance meter into the external auditory canal and the middle ear The patient was then asked to swallow a sip of water repeatedly as a rule ten times The equalization of the negative pressure induced into the middle ear occurred in stages either fully or partially to a certain level and this residual negative pressure was recorded

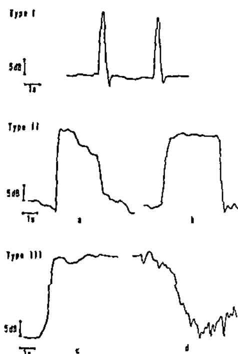


Fig 1 Different sonotubometric wave patterns For further details see text

A tympanogram was made and the impedance changes caused by forced respiration through the nostril at the examined side were also recorded by the above mentioned impedance measurement equipment in most cases The hearing was estimated with the pure tone audiogram

RESULTS

Nearly all the patients (24) had abnormal functioning of the Eustachain tube as examined by sonotubometry The tubal opening recordings measured in the frequency of 7 kHz were divided into three groups according to the nature of opening Fig 1 shows samples of the different sonotubometric wave patterns

Type I function is characterized by a relatively sharp peak of increase in the sound pressure level during swallowing It describes the normal finding of the Eustachian tube function (Virtanen 1977, 1978)

'le I *Twenty five cases of patulous Eustachian tube*

		Symptoms in ear				Results of examination			
Age	Sex	Sensation of fullness	Autophony	Respiratory sound	Relief by lying down	Drum moves with respiration	Sonotubometric curve type	Variations in impedance with respiration	Residual negative middle ear pressure (mmH_2O)
48	♀	+	+	+	−	−	I		
30	♀	+	+	+	+	−	II		
83	♂	+	+	+	+	+	II		
57	♀	+	+		+	−	II	−	
36	♀	+/−	−	−	−	+	II	+	
56	♀	−	+	−	−		II	+	
38	♂	+	−	−	−	+	II	+	
52	♀	+	+	+	+	+	II	−	
[illegible]7	♂	+	−	−	−	+	II	+	
35	♂	+	+	+	+	+	II	+	
40	♂		+	+	−	+	II	+	
29	♀	+	+	+	+	−	II	+	
36	♀	+	+	−	+	−	II	−	
30	♀	+	+	+	+	+	II	+	
31	♀	+	+	+	+	−	II		
46	♂	−	−	−		perfor	II		−100
27	♀	−		−		perfor	II		0
38	♀	+	+	−	+	+	III		
51	♀	+	+	+	+	+	III	+	
53	♀	+	+	+	+	+	III	+	
[illegible]8	♂	−	+	+	+	−	III	+	
38	♂	+	+	+	+	+	III	+	
27	♂	+	+	+	+	+	III	+	
[illegible]8	♀	+	+	+	+	+	III	+	
30	♂		−			perfor	III		−200

ı the second type (II) the tube opens very
l on swallowing and for some time after
ds and closes little by little (Fig 1*a*) or ab-
tly with or without further swallowing (Fig

n the third type (III) the Eustachian tube
ns well on swallowing and remains open
a long time or is continuously open (Fig
and it may be closed by bending the head
ward (Fig 1*d*), by lying down and/or by
ffing

able I shows the analysis of symptoms and
ngs related to the tubal function for all
es In 6 patients both ears were involved
ly one person's (patient 5) symptoms varied
m ear to ear The results of examination
re same on both ears of all these 6 patients
first 17 patients are those who showed
e II of sonotubometric curve except one
e (patient 1) who had the normal sono-
tubometric curve The fact that she had had a common cold a week ago may be taken into account and probably the swollen mucous membrane was still resulting in a normal tubal opening Two patients (16 to 17) had a dry, central tympanic membrane perforation

None of the 15 patients with intact eardrums had the symptoms of the patulous tube continuously but at most for a few hours a day and not always every day Three patients did not feel the sensation of occlusion or fullness of the ears, 3 out of these (15) were not annoyed by autophony and 6 patients did not hear their own respiration in their ears Only 9 patients obtained relief by lying down and placing the head down between the knees In 8 patients the drums were seen to move (examined by Siegle s otoscope) with respiration at the commencement of the examination According to the sonotubometric curve the

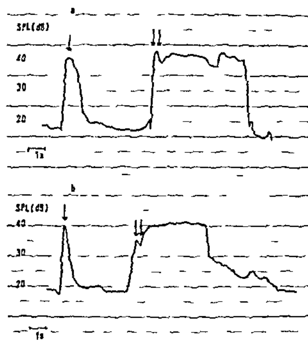


Fig 2 Two individual sonotubometric examples of tubal *opening during* swallowing (↓) and by means of yawn*ing* (↓↓) *Upper curve (a)* the patent tube is closing voluntarily and abruptly after yawning, and lower curve *(b)* *it is closing little by little* by bending the head forward

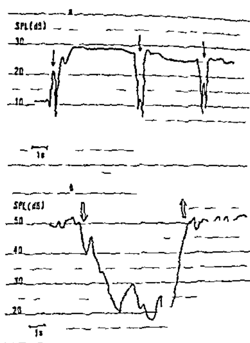


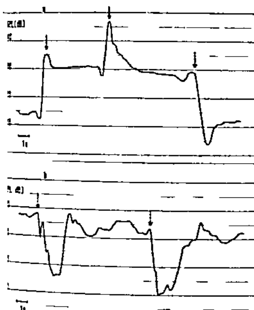
Fig 3 The individual sonotubometric curves of 2 pat[illegible] the act of swallow [illegible] patent tube closes on bending the head between the knee and opens on raising the head again The thick arrow mark the direction of head moving

tachian tubes in these 17 ears opened after swallowing for the time of 1~13 sec closing little by little or abruptly (Fig 1, type II) Ten of the first 17 patients were able to open their tubes easily by means of yawning and to close them voluntarily (Fig 2*a*) or by bending their heads forward (Fig 2*b*)

Nine patients (8 to 16 Table I) had had otitis media previously, and even tympanostomy tubes were used in one patient One patient (17) had had a fracture of the base of the skull as the result of an accident

In the remaining 8 patients (18 to 25) the sonotubometric curves were of the type III (Fig 1), i e the Eustachian tube was open continuously or for a long period of time and did not close during swallowing (Fig 3*a*) Of these patients with intact eardrums only one stated that the ear was not occluded Each one of these patients heard his own voice (autophony) and all of these patients were able to obtain relief by lying down or bending the forward (Fig 3*b*) One patient did not hear h own respiration in the ear and in one pat drum movements with respiration were n seen Of the 8 patients with type III sonc tubometric curve, 4 had learnt to get rid of symptoms by means of a quick inspira through the nose (sniffing) intermittently 4*a*), whereas some did not succeed in the attempt to close the tubes by sniffing (Fig 4*b*) Three (18 to 20) out of the remaining 8 tients had a history free of ear disease and had had otitis media several times in or more recently, tympanostomy tubes w even needed for one patient One patient (2 had had a fracture of the base of the skull bu no ear trouble prior to that

None of the 3 patients (16, 17 and 25) w an eardrum perforation had any symptoms patulous tube The pressure equalization t was also made in these three ears One



; 4 Two nd vidual sonotubometric curves of the pa
" u

' ng( ) Lower curve (b) the cont nuously patent tube
ses only for a moment by means of sniff ng (↓)

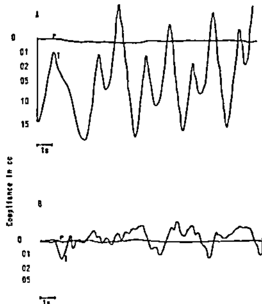


Fig 5 The variations in impedance of the ear with res p rat on on two d fferent patients (*r*=right ear normal tubal funct on *l* left ear patulous tube) For further de tails see text

le to equalize the negative middle ear pres re partially to levels of -100 mmH_2O only ile another was not able to equalize it at all)wever both these ears had an abnormally tent Eustachian tube on examination by notubometry the types of curves were II d III The third ear with tympanic mem ane perforation was able to normalize the gative pressure introduced into the external r canal and it had sonotubometric curve II

Four patients (18 to 20 and 24) reported too it they often suffered from a sensation of oc ision in the nose and/or a nasal discharge In e patient even allergy alone was suspected being the main cause The otologic exami tion was essentially negative and it revealed ar nasal passages in all these patients Two tients voices were heard to be decidedly ponasal but the phoniatrician did not re gnize any disturbance of the velar function articulation

The hearing level of all these ears with intact eardrums was within 20 dB of the ISO stan dard reference threshold at the frequency range of 500–2 000 Hz They had as a rule the normal shape of tympanogram too With the aid of an impedance measurement the varia tions in impedance could be observed syn chronously with respiration in nearly all pa tients The amplitude varied greatly not de pending in any way on the kind of tuba aperta that existed as shown in Fig 5 where the pa tient A (10) had the sonotubometric curve type II and the patient B (19) had the curve type III denoting the continously open tube

COMMENT

There are many people who have patulous tubes and who suffer from the symptoms either continuously or periodically (Munker 1977) The symptoms are variable because the patency is not always constant and it may vary from a mild unilateral form to a con stantly open bilateral form

The symptoms in this material also varied a great deal, and thus the diagnosis of tuba aperta could not be reliably founded on them alone The patients with a tympanic membrane perforation did not suffer from the symptoms of the patulous tube at all This is in accordance with the suggestion of making a myringotomy with tube insertion into the middle ear in order to offer improvement or complete relief (Suechs, 1960, Thaler & Yangisawa, 1966)

The analysis of the sonotubometric findings of this work showed that the patients with a patulous Eustachian tube had some typical characteristics in their wave patterns (Fig 1) Thus, apart from one patient, the diagnosis could be confirmed by means of the sonotubometry This patient did not have a patulous pattern of tubal opening during swallowing which evidently was due to a nasopharyngitis that she had had just a week before

By measuring the variations in impedance of the ear during forceful respiration the diagnosis might be established, as described earlier by Metz (1953) too However, the movements of the tympanic membrane are not always present at the moment of the examination and thus a false negative result can be obtained, as on 4 patients in this material Measurements of impedance variations did not correlate well with the results of sonotubometry concerning the type of the patulous tube

In two ears out of these three with an eardrum perforation the pressure equalization test was inapplicable to the evaluation of tubal function Only one patient could equalize the negative intratympanic pressure fully, even though all the tubes were abnormally open It is evident that the pressure equalization test does not give correct information about the patency of the tube, because a negative middle ear pressure can cause the locking phenomenon of the tube and produce an obstruction that the muscle activity of the tube is not able to overcome (Virtanen 1977, Palva et al, 1978) Thus on the ears with a drum perforation the sonotubometry alone gives a quite distinct objective impression of the permeability of the tubes It may be worth noting because after the healing of the eardrum symptoms of the patulous tube are more li[illegible] to affect these persons, who have a defiency in the closing of their Eust[illegible] tubes According to Ekvall (1977) these patients often try to close their tubes by sniffing and thus cause a medial displacement [illegible] the eardrum

Two patients (17 and 21) were inj[illegible] result of an accident, with a fracture of base of the skull passing through the [illegible] They had had no ear trouble before thus fracture was the apparent etiological facto[r] and the pathological basis for their condition

In this material there were 9 (36%) pat[ients] who had suffered many otitis attacks and con[-] current middle ear effusions or discharges [in] their previous history On the basis of this etiology of the recurrent otitis media could some of the cases be attributable to the fail[-] ure of the Eustachian tube to close for [con-] siderable periods of time Later, by followin[g] the infection of the middle ear, [illegible] ical changes may develop on the mucosa of th[e] Eustachain tube and the tube can then partially or totally obstructed and the need fo[r] prolonged use of tympanostomy tubes arise (cases 11 and 22) Aschan et al [illegible] assumed that the sniffing repeated continual several times a day, may be the cause of at[e-] lectatic tympanic cavities in these patients but this will require further investigation

Three patients had feelings of discharge an[d] obstruction in their nasal cavity similar to [illegible] symptoms of a common cold One patient o[f] of these 3 had been suspected of allergic mar[ni-] festation and had been examined thorough[ly] but with negative results None of these [illegible] the nasal obstruction and distinct d[illegible] from the nose at the examination All the[ir] tubes were open continuously or for lo[ng] periods of time (sonotubometric type III) they were perpetually trying to sniff Two pa[-] tients had inadvertently learnt to avoid nasal respiration preventing by velar fu[illegible]

nasal resonance from extending through tube into the middle ear. Similar hypo-sality of speech caused by the patulous tube s also been described before by Landes 967), and Batze & Parker (1971).

ZUSAMMENFASSUNG

werden 25 Patienten (31 Ohren) mit pathologisch klaf den Ohrtrompeten vorgestellt und die Symptome ana-iert Alle Ohren wurden mit Hilfe der Sonotubometrie ersucht und es konnten zwei verschiedene Öffnungs-en bei der offenstehenden Tube unterschieden werden Schwankungen der Impedanz des Ohres bei der Re-ation wurden in fast allen Ohren aufgezeichnet, und Ohren mit perforiertem Trommelfell wurden auch mit fe der Luftdruckausgleichsmethode untersucht Nur Sonotubometrie zeigte deutlich die offenstehende be in allen untersuchten Ohren Sie ist somit eine lichbare zusatzlicht Methode zur Diagnose des abnor-len Offenseins der Ohrtrompete und zum Verfolgen der handlungsergebnisse, wobei dann die Beurteilung der handlung keinen Raum fur Vermutungen laßt

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TUBAL CLOSING FAILURE IN RETRACTION TYPE CHOLESTEATOMA AND ADHESIVE MIDDLE EAR LESIONS

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Abstract In cases of retraction type cholesteatoma and related conditions it has been observed that the Eustachian tube may show a reduced ability to withstand respiratory pressure variations The reduced resistance of the tube may play an important role in the development and course of middle ear disease In the present study 42 subjects who had middle ear disease of this type and confirmed habitual sniffing behaviour were examined for symptoms and signs that would indicate a patulous tube Different degrees of malfunction were recognized in the presence of *total* closing failure with an intermittently free transfer of respiratory pressures to the middle ear the sniffing procedure maintains a negative intratympanic pressure The condition is identical to the patulous tube state in the classical sense In *relative* closing failure there is no free pressure transfer and no patulous tube symptoms Sniffing may however cause a repeated evacuation of the middle ear space The results of this study cast doubt upon the conventional views on tubal malfunction in middle ear disease and indicate a need for reconsidering the etiology of these lesions

The physiological role of the Eustachian tube is usually thought of in terms of different components

(1) Ventilation of the middle ear space and of the air cell system is regarded to be the most important function The tube is opened briefly by muscular contraction during deglutition, permitting an intermittent passage of air Any volume deficit caused by the gas exchange in the middle ear, and any change of atmospheric pressure, are thus compensated for A pressure difference between the lateral and medial sides of the tympanic membrane is thereby eliminated

(2) In the passive, closed position the tube protects the middle ear against sudden pressure changes in the nasopharynx caused by respiratory activity and phonation W closed, the tube also protects the middle ear against infection which might ascend from the nasopharynx

(3) The mucociliary function takes care o clearance of mucus and foreign material in cluding bacteria

That a close interrelation exists between th different components of the Eustachian tub function is obvious but investigations of th function have dealt almost exclusively wi ventilation with respect to anatomical an functional patency An impaired ventilator ability is believed to be a contributing fac in the development of different diseases of th middle ear It is also believed to be causativ in failure of healing after reconstructi ear surgery, and in recurrent cholestea Clinical tests used at present are based on th assumption that the capacity to equalize art ficial pressures is crucial For example the a piration—deflation method described by F berg (1966) has been used to a large exten with individual variations in the evaluation o "tubal function" Current methods have re cently been reviewed by Cohn (1977) wh questions the relevance of the aspiration as far as the physiologic function of the tube i concerned and concludes that the test is no suitable for predicting the outcome of tym panoplasty This is in agreement with findings of Ekvall (1970) and Palva & Kan (1970)

When a suitable surgical technique is us in myringoplasty, there is no correlation

tween the aspiration test result and postoperative healing There is thus an obvious need for examining the tubal function test To test the normal function of the tube under realistic circumstances may actually call for a rather different approach, an integral part of which would be to remove much of the present emphasis on the ventilatory capacity

Closing Action of the Tube

According to Elner (1977) the function of the tube is not ventilatory in a strict sense The extremely small quantities of air which normally pass through the tube do not change the gas composition of the middle ear space significantly A better definition of the function of the tube would be pressure regulation, or pressure control Considering the rather abrupt respiratory pressure changes which take place in the nasopharynx, it seems more apt to focus on the closing ability of the tube

The patulous tube condition has been referred to in a number of papers Since the condition involves an apparent "hyperfunction", it has been set clearly apart from the tubal problem in middle ear disease A good review of the literature and of the symptoms of this condition was published by Miller in 1961 In his material and in others, are included several cases of atrophic tympanic membranes Miller concludes that the state is much more common than is appreciated, depending to a great extent on the difficulties which the patients have in describing their symptoms

Moreover, the patulous tube state is easily confused with its apparent opposite, the stuffed tube with serous otitis media It should be noted here that the two clinical entities are perhaps not mutually exclusive Munker (1977) has noted a patulous tube in many cases of cleft palate, and Zollner (1942) has often observed a patulous tube in cases of both serous otitis and chronic otitis media

In persons with a patulous tube, negative middle ear pressures are created by repeated, forceful nasal inhalations, forcing the tube to close It is well known that such persons very often use a sniffing procedure to suppress their symptoms Possibly, the self induced underpressures might cause structural lesions in the middle ear

The following study was initiated as a result of a clinical observation A young woman of 28 had bilateral attic cholesteatomas, collapsed tympanic membranes and effusions in the middle ears In the preoperative period the patient was instructed to ventilate her ears actively by Valsalva inflations She refused firmly Instead, it was discovered that she performed reverse Valsalva manoeuvres continuously in order to avoid autophonia, respiratory drumhead flutter and hyperacusis In spite of great efforts at persuading her, the patient still refused to try Valsalva inflation It was eventually possible to convince her to abandon the reverse Valsalva manoeuvres, however From that day, both middle ears became spontaneously air filled without effusion, and her hearing has remained good for the 5 subsequent years

In this case there was evidently a defective closure of the tubes, which had caused the patulous tube symptoms and the reactive behaviour The opening action of the tubes proved to be sufficient when the self induced evacuation of the middle ear space was omitted The same impairment was soon found in several other cases having middle ear disease of similar type Ultimately, it was suspected that a direct, causal relationship might exist between the failure of the tube to close and the development of chronic middle ear disease, and that the relationship might constitute a generalized etiological principle In the present study the attention is focused on the reduced ability of the tube to remain closed in the resting position These aspects have been presented preliminary by Ekvall & Magnuson (1976, 1977), Magnuson & Ekvall (1976), Aschan, Ekvall & Magnuson (1977) This study is a systematic analyse of anamnestic data and otomicroscopic findings in a selected material, easy to adapt in clinical practice

Table I

Healthy ear	4
Healthy ear post comb appr tympanoplasty	3
Recurrent serous otitis media only	2
Atrophy of pars tensa only	10
Deep retraction of pars flaccida only	8
Deep retraction of pars tensa and pars flaccida	17
Retraction type cholesteatoma not operated	21
Advanced adhesive otitis	2
Perforated retraction	1
Radically operated ear	9
Complication post comb appr tympanoplasty	7

MATERIAL AND METHOD

The present material consists of 42 subjects who were either treated conservatively for middle ear disease, were awaiting surgery, or had previously undergone middle ear surgery on one or both ears The subjects were selected because they had a pronounced habitual sniffing behaviour The age range was 14–65 years, mean age 39, median 41 5 years No young children were included, since they cannot describe their symptoms accurately 14 subjects were female and 29 were male In most cases the middle ear disease was bilateral, only 4 had unilateral disease The diagnoses in all 84 ears at the time of the investigation are given in Table I It is seen that there were no cases of central perforation in the material

The lesions range from subtle atrophic changes of the tympanic membrane, to deep retractions, cholesteatoma in retraction pockets, 'spontaneously operated cases with a wide destruction of the medial canal wall and labyrinthine fistula, and finally to advanced adhesive otitis with a totally obliterated middle ear space, save for a tiny air bubble in the tympanic orifice of the Eustachian tube

A long history of purulent drainage was present in 26 of the subjects The severity of diseases in the group may be indicated by the fact that 19 of the subjects had earlier undergone middle ear surgery a total of 32 surgical procedures and revisions The material includes 7 cases of recurrent retractions post CAT procedures One of these had recurrent cholesteatoma, 2 had a late penetration of the columella through the healed fascia graft and one had a late spontaneous hemotympanum At the time of the investigation 9 of the subjects were being treated for middle ear effusions by ventilating tubes

All ears were examined by otomicroscopy and by simple functional tests The subjects were questioned systematically about symptoms, provocative factors, and sniffing

RESULTS OF INTERVIEW

A *Autophonia*

A blocked sensation in the ear is described by most patients and might easily be mistaken for serous otitis media These patients frequently have effusions, which might add further confusion When the patients were questioned in detail, a common picture of a specific type of autophonia arose One s own voice seems hollow, as if one were speaking into an empty barrel, it seems too loud roaring and distorted The nasal speech sounds break through as do the palatal click sounds, and breathing sounds The symptoms are of very short duration only a second or two, and are instantly switched off" by sniffing Twenty nine of the subjects had autophonia and 90% of these patients had it in the form of short recurring episodes

	n	%
autophonia	29	69
no autophonia	13	31
total	42	100

Course of autophonia

	n	%
short recurring episodes	26	90
single episodes	1	3
lasting autophonia	2	7
total	29	100

The autophonia could be evoked by certain factors in 19 of the subjects The remaining 10

subjects could not indicate any provocative ors

ovocative factors

Physical strain, like lifting heavy objects, running playing tennis and soccer 14 subjects mental strain and stress 7 subjects, ironmental temperature change, like going outside in cold weather 6 subjects

B *Drumhead Flutter*

Movement of the tympanic membrane synchronously with respiration was a common symptom in this group of patients It is described as a feeling that the drum is loose and will not come to a resting position Clicking or pumping sounds arise in the ear upon breathing The symptoms are both tactile and acoustic The flutter is instantly switched off by sniffing Twenty seven of the subjects had drumhead flutter, and in 82% of them it appeared as short recurring episodes

	n	%
drumhead flutter	27	64
no drumhead flutter	15	36
total	42	100

Course of drumhead flutter

	n	%
short recurring episodes	22	82
isolated episodes	3	11
lasting drumhead flutter	2	7
total	27	100

The flutter could be evoked by certain factors in 21 of the subjects The remaining 6 subjects could not associate the symptoms with any specific events

Provocative factors

Physical strain 16 subjects
Mental strain 6 subjects
Temperature change 5 subjects

C *Hyperacusis*

Hyperacusis was described by 17 subjects This disturbance is apparently paradoxical in view of the fact that most subjects had impaired hearing with different degrees of air–bone gap In many cases it was found that not only the patient's own voice but also environmental sound was heard too loudly With "drums out" after spontaneous equalization caused by swallowing, the sound appears too loud, echoing, sharp and distorted With "drums in" the hearing is more comfortable, natural and distinct Hyperacusis is also of short duration, and is suppressed by sniffing in these patients

D *Common Colds*

Patients with a patulous tube can generally relieve themselves of their symptoms by lying down, which was confirmed in all but two In the majority of the present cases, the outbreak of a common cold was not followed by relief but was often associated with an enhancement of symptoms, including an increased need for sniffing In the late stages of the infection the autophonia, drumhead flutter and hyperacusis became less annoying, but at the same time the hearing was often more impaired than usual

Symptoms at the outbreak of a common cold

	n	%
autophonia worse	15	52
no change	13	45
improvement	1	3
total	29	100
	n	%
drumhead flutter worse	14	52
no change	12	44
improvement	1	4
total	27	100

E *Voluntary Suppression of Symptoms*

Voluntary suppression of symptoms by means of sniffing was claimed to be habitual and to date back as long as the subject could re-

member in 25 of the 42 subjects in this material, or 60% Occasional sniffing was claimed by 13 subjects, 30% Four persons said they were not conscious of any sniffing, though an obvious sniffing behaviour could be observed

In 29 cases sniffing only was used, and in 7 cases it was used in alternation with reverse Valsalva manoeuvres In 6 cases, only reverse Valsalva was used to suppress the symptoms

F *Reasons for Sniffing Procedure*

Several subjects gave more than one reason for their sniffing habit

	n	%
no reason	5	12
autophonia	25	60
drumhead flutter	20	48
hyperacusis	17	40
noise protection	12	29
to hear better	16	38
other reasons	8	19

Among 'other reasons' was the need to relieve high frequency tinnitus, which was mentioned by 2 subjects As possibly contributing factors were allergic rhinitis or other nasal problems in 11 cases, and significant occupational noise exposure in 21

G *Active Ventilation for Hearing Improvement*

The majority of the patients studied were able to increase their hearing acuity by Valsalva inflation when listening to weak sounds Not all utilized this possibility however Thirty-three stated that they could improve their hearing by inflation, but only 17 were inclined to do so When evaluating this finding it should be noted that all subjects had been instructed earlier and motivated repeatedly to carry out active ventilation in connection with the treatment of hearing loss and middle ear effusions When interviewed in the present study, 50% claimed to be unwilling to perform ventilation Several subjects stated more than one reaso for their unwillingness

	n	%
no statement	18	43
ventilation would bring on		
autophonia	14	33
drumhead flutter	1	2
hyperacusis	12	29
pain	3	7
other reasons	7	17

Other adverse effects experienced by subject to such an extent as to render them unwilli to carry out ventilation were sudden vertgo i 2 cases and painful pressure in the eyes in case

RESULTS OF CLINICAL EXAMINATION

A *Valsalva Inflation*

Patients were instructed to perform Valsalv inflation under visual control via the otomicr scope As mentioned some subjects were un willing to perform inflation This was espe cially true of the younger individuals The pa tient's attitude toward performing Valsalva in flation and, once willing his way of doing so provided fairly good indicators of the present of closing failure Some persons merely mar the inflation by keeping the glottis close during the procedure It was usually very eas to obtain passage when the right techniqu was used Even when assuming a supine pos tion on the examination table the patien could generally perform the inflation wi ease In this position many normal subjec have difficulty in inflating their ears Charac teristic for the present patients was that th tympanic membrane first bulged due to the in creased pressure, and then collapsed again seems that the tube cannot maintain any pos tive pressure within the middle ear in the cases In 10 ears the presence of a ventil tion tube or a perforation made the test i relevant

Passage in Valsalva inflation

	n	%
negative	6	8
positive great resistance	7	10
positive easy passage	6	8
positive easy passage in supine position	55	74
total	74	100

B *Sniff Test*

The sniff test was carried out during oto-microscopic examination After Valsalva inflation the subject is asked to occlude one nostril with the finger and to perform sniffs with increasing force If the tympanic membrane is seen to retract instantly, the test is considered positive Sometimes the drumhead flutter caused by slow or forced respiration may be observed Often the subject has to sit up in order to perform the Valsalva inflation If he still does not succeed a Politzer inflation is performed The subject is then asked to lie down on the examination table again, and told to make sure that he does not sniff until the microscope has been focused Sometime the test is positive in the sitting position but not in the lying position It is important that the subject does not spend unnecessary time in the supine position The increased hydrostatic pressure makes the tube close very rapidly In the presence of a perforation, a ventilating tube or a stiff and tympanosclerotic tympanic membrane the test is not relevant In the present material the test was irrelevant in 1? ears

Result of sniff test

	n	%
positive test	59	82
negative test	13	18
total	72	100

CLINICAL PRESENTATION OF CLOSING FAILURE

A typical case in this material is a 40 year old male subject with bilateral attic retractions or cholesteatoma, a patulous tube condition and a confirmed habitual sniffing habit for many years Most of the subjects stated that they had had the symptoms, as well as the sniffing habit, for as long as they could remember Some of the subjects could give lucid accounts of childhood experiences regarding these matters The patulous tube condition thus does not seem to be limited to advanced age, weight loss, hormone treatment or other general conditions Erect posture and stress situations of various kinds seem to provoke the symptoms in the present cases

A pronounced variability between different patients is indicated by the present findings Furthermore, when subjected to repeated examinations, one and the same individual will display different impairment pictures On one occasion, a total closing failure with a free respiratory drum movement could be found A common cold may cause the tube to be totally blocked, and a few weeks later the closing failure may turn up again There is thus a very pronounced intra individual variation The malfunction is not a static condition, but rather, rapid change seems to be inherent The tubal malfunction may be described by its degree and duration, and all combinations seem to be possible The term ‘ tubal closing failure ’ may then serve to cover various conditions involving an impaired resistance of the tube Some systematization may be supplied by dividing the conditions into the following clinical groups

A *Total Closing Failure Constant Type*

Such cases are detected accidentally, since there are no symptoms A person can obviously get used to this type of closing failure At repeated examinations the drum is seen to move synchronously with respiration, a manifest condition of a wide open tube which may be detected easily by otoscopy If no progressive middle ear disease is present, there is no need for treatment In order not to evoke

his anxiety, the patient should be informed that the findings do not indicate disease

B *Total Closing Failure, Intermittent Type, Non suppressed*

This condition is met with more commonly, since the patient is alarmed by the intermittent onset of symptoms Because positive findings may not be present at the time of examination, the nature of the disorder is easily misinterpreted, and the patient may seek medical advice repeatedly The condition seems to produce very pronounced symptoms, by virtue of its being intermittent Habituation is therefore not possible

There are difficulties in tuning the voice to a suitable loudness level When the tube is open, the autophonia causes a distressing and constantly varying error in the natural feedback of speech and hearing, which may lead to a severe neurosis Drumhead flutter, the feeling that the tympanic membrane moves and flutters as one breathes is also trying The membrane may move in a distressingly irregular manner, depending on changes in depth and velocity of respiration Most of these cases can be diagnosed easily if attention is paid to the case history

Information to the patient on anatomy and function in common terms is the primary treatment This provides a rational explanation for the symptoms, and will often relieve the patient's anxiety The symptoms are evoked by stress situations and are usually of only some minutes' duration When told that such symptoms are quite common and that they do not indicate disease, the individual will tolerate them more readily For the most part no other treatment is necessary In the case of more protracted symptoms, the insertion of ventilating tubes may eliminate the symptoms or relieve them significantly

C *Total Closing Failure Intermittent Type, Suppressed*

This condition constitutes the main issue of the present paper Sniffing is used habitually to suppress the symptoms This procedure i well known in patulous tube cases but the re lation to retraction type cholesteatoma doe not seem to have been recognized By a force nasal inhalation or a reverse Valsalva man oeuvre a low middle ear pressure is induced and is maintained for some time because of th valve action of the tube The subject is thu briefly relieved from his symptoms when th tube is locked Upon swallowing the tube ma easily open again causing the autophonia t reappear The good equalizing ability of th tube is thus maintaining the state and th events are repeated in a cyclic fashion ove and over again

Presentation

In the present cases the sniffing habit seems t have been acquired early in life The subjec has found a way to cope with his symptom and he usually seeks medical care not becaus of autophonia, but because of middle ear di ease However, this is often not discovere until specific symptoms of chronic otitis medi arise Hearing frequently remains good an discharge, pain and other complications ar late symptoms At the time of admittance th impressive otoscopic findings attract atten tion The specific tubal malfunction is not ex pected and is therefore easily overlooked Fu thermore, the symptoms may have been pre sent for many years, but habitually suppresse by sniffing on an everyday basis Hence th patient may not even be aware of any symp toms himself

Symptoms

Many patulous tube cases have normal hearin according to routine audiometry Even so th subject experiences a severely disturbed dis torted hearing When questioned some ca give a full account of their symptoms whil others merely describe a vague feeling of unea siness Upon swallowing or yawning a pop ping sound may be experienced and the ear i said to feel ' blocked The automatic reac tion of the individual is to sniff The patien

has a very strong desire to make the drums come to a stand still in the retracted position and the hearing is said to be better after sniffing

Objectively an impaired hearing exists in the majority of the present cases but hearing loss is not a primary complaint The patient may find *hyperacusis* to be the most disturbing symptom Sometimes it is the only one A probable explanation for this paradoxical symptom is habituation These subjects are primarily sniffing in order to suppress the autophonia and drumhead flutter and seem to accept a certain impairment of the hearing caused by the low intratympanic pressure As they become more and more used to it they come to consider it as their natural hearing By habituation the experience of loudness may transform itself into a symptom of its own With drums out the subject experiences environmental sounds as strange unpleasant unreal sharp and non clear When the sound image is changed suddenly by spontaneous tubal equalization a compulsory stimulus for a sniffing reaction is provided even though the subject may be free from autophonia at the time

The condition is especially troublesome for the subject who has a noisy occupation If he has to speak loudly or to shout in order to make himself heard the autophonia and hyperacusis become intolerable A certain conductive impairment is advantageous in a noisy environment and in the case of closing failure the subject makes use of his unique ability to protect himself Sniffing is the only way to master the situation

Obviously a vicious circle develops where secondary conditioning makes the subject sniff unceasingly even during periods when there is no total closing failure and no autophonia These subjects are therefore approaching the next category of relative closing failure

Diagnosis

The presence of intermittent autophonia drumhead flutter and hyperacusis as well as the sniffing habit may be revealed if the patient is questioned in detail In many cases the diagnosis is apparent at the first visit if attention is paid not only to the middle ear disease but also to a careful penetration of the patient s symptoms and to observing his behaviour during the examination The sniff test should be carried out routinely and repeated examinations are necessary whereby many of these cases may ultimately be diagnosed objectively

Treatment

How to treat closing failure as such is not settled although different methods have been tried in the past Treatment should be aimed at the causal factor which is still unknown It might be a defect in the tubal secretion which might not be sticky enough to keep the mucous surfaces of the tubal slit firmly glued together in the closed resting position It seems that viscous fluid is very effective in blocking the tube while serous fluid is not In the majority of the present cases there is retraction of the tympanic membrane and middle ear effusions have been observed in several Typically the effusion is thin and watery with some air bubbles anteriorly near the tympanic orifice of the Eustachian tube It might thus be possible to classify the common serous otitis media together with the present cases

The appearance of a viscous effusion a perforation of the tympanic membrane or an interruption of the ossicular chain might contribute to extinguishing the sniffing by relieving the patient from patulous tube symptoms There is some evidence that some patients suffering from recurrent effusions are actually utilizing this effect by sniffing vigorously until the appearance of effusion relieves them from symptoms The lesion is induced and maintained actively and spontaneous healing is counteracted

Because of the confirmed reactive behaviour treatment is definitely no easy task In some cases it is possible to get the patient out of his habitual sniffing He will find that the

patulous tube symptoms are present for short intervals only, e g in the presence of physical or mental strain, temperature change, a common cold or other circumstances of stress He may find that when the sniffing is avoided the symptoms are not so very disturbing after all However, in many cases it is seemingly impossible to convince the patient to abstain from his sniffing habit

The insertion of ventilating tubes is the only simple and instantaneously effective treatment available The tubes will cause a rapid disappearance of effusion, render underpressures impossible, often relieve the patient from his patulous tube symptoms, and also seem to influence the patient's sniffing behaviour The hyperacusis may be exaggerated during the first few days and ear protectors may have to be used for some time in order to attenuate environmental sound Unfortunately, a relapse into the old habitual sniffing behaviour seems to take place very easily when the tubes are extruded

D *Relative Closing Failure*

This term might be used to denote a state where the respiratory pressure variations are not freely transferred to the middle ear space The tube is thus not patulous in the strict sense of the word Negative nasopharyngeal pressures are given passage, while attempts to ventilate the middle ear by Valsalva inflations may meet with great difficulties The pronounced one-way valve action of the tube seems to be decisive In apparent cases the valve action is extreme enough to make Valsalva inflation impossible A nasal inhalation, without being forced, may induce a retraction of the tympanic membrane In less obvious cases the tube appears semi blocked, and only when the tube is subjected to more excessive negative pressures does a pressure break through occur

Evidently, a habitual sniffing behaviour is not developed in such cases nor is it a necessary precondition for maintaining a negative middle ear pressure The pressure may b reduced occasionally, as during snoring in common cold At present, relative closing fail ure seems to represent a wide and rather un known field It might be a very common state but it is a most difficult condition to diagnos objectively No acoustical symptoms ac company the relative closing failure and diagnosis cannot be made from the case his tory The subject may have a positive snif test occasionally, but the state seems to var from time to time just as the two previou ones do

Relative closing failure has been found in some cases of glue ears and also in most ad vanced cases of chronic otitis media Instruc ting the patient to perform Valsalva inflation intermittently seems to be successful trea ment in some cases However, when the infla tion is performed, air may escape spontane ously, because the tube cannot maintain positive pressure within the middle ear Th use of ventilating tubes is frequently nece sary, especially in children

It should be noted that although the midd ear disease is usually bilateral the severit of the disease frequently takes on quite di ferent manifestations Not infrequently th *least* affected ear is found to have a closin failure of the suppressed C type , while th most severely diseased ear is found to have totally blocked tube at the time of examina tion The functional defect of the tubes is thu asymmetrical, seemingly of opposed types I such cases there is some evidence to indica that one ear may be the 'leading one causin intermittent patulous tube symptoms as we as habitual sniffing The 'second ear where n closing failure is demonstrated upon examina tion might be influenced by the sniffing an be blocked because of it A relative clos n failure may thus be present It has been ob served that a ventilating tube inserted in th 'leading ear may improve the state of th 'second one Such information might be o significance in surgery for chronic middle ea disease

CONCLUSIONS

patulous tube state is not a uniform or static condition When analysed more closely, it is found to give rise to quite different clinical pictures some of which will elude diagnosis if they are not searched for actively It might be found that the tubal malfunction of interest in chronic middle ear disease is a failure not of opening but of closing

In the present cases the middle ear space is evacuated from the nasopharynx and, in a literal sense the tympanic membranes are retracted because the subjects retract them The questions are why and how do they do so, what are the consequences, and what is the significance of this in a non selected population? In this paper the patients' own accounts of their experiences are used as point of departure The present material was included in an investigation in which objective measurements were carried out by pressure recording The results will be dealt with in a paper to follow

The theory advanced here is that chronic adhesive middle ear lesions and retraction type cholesteatoma may develop as a reaction to certain types of tubal closing failure The under pressures which are induced may be responsible for retraction and atrophy of the tympanic membrane, effusion of fluid, and a progressive atelectasis of the middle ear space with involution and sclerosis of the mastoid air cell system By permitting the accumulation of keratin debris in retraction pockets the same process may be indirectly responsible for the development of cholesteatoma Since the process starts early in life, the original tubal malfunction will not necessarily be permanent When irreversible damage to the tympanic membrane has been induced, the development of cholesteatoma with secondary infection and further damage to the ear may proceed autonomously In certain cases the tubal malfunction may remain or reappear, leading to a tendency to postoperative effusion, new retractions of the grafted tympanic membrane, and recurrent cholesteatoma

ZUSAMMENFASSUNG

Bei Erkrankungen des Mittelohrs hat man sich früher ganz auf die Öffnungsfähigkeit der Tube konzentriert In vorliegender Arbeit wird im Gegensatz die Schließungsunfähigkeit der Tube bei Retraktionscholesteatomen und ähnlichen Zuständen als krankheitserregend dargestellt Vier verschiedene Formen des Funktionsdefekts sind definiert welche klinische Variationen von klaffender Tube in klassischer Bemerkung darstellen Über Resultate von Druckmessungen in Retraktionsfällen wird in einem folgenden Artikel berichtet werden

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SUDDEN DEAFNESS IN RELAPSING POLYCHONDRITIS

A Scanning Electron Microscopy Study

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Abstract The left ear of a 57 year-old female who suffered sudden deafness during the course of relapsing poly chondritis was examined under a scanning electron microscope Marked degeneration of the organ of Corti in all turns and dislocated and encapsulated tectonal membrane were found in the cochlea Marked decrease in number of the sensory cells in the utricular and saccular maculae and total loss of sensory hair bundles in the ampullary cristae of the semicircular canals were seen in the vestibule These findings strongly suggest that the cause of sudden deafness in this case might be viral The usefulness of scanning electron microscopy in human temporal bone pathology is stressed

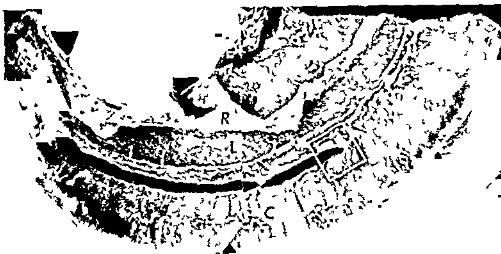
Though sudden deafness is not a rare occurrence in the otolaryngologic clinic, studies on inner ear pathology are still scanty Inner ear findings in cases of sudden deafness of unknown etiology showed changes similar to those found in known viral labyrinthitis (Beal et al , 1967, Schuknecht et al , 1973) and, though fewer in number in vascular disorder (Gussen, 1976) and in probable cochlear neuronitis (Ishii, 1977) The temporal bone studies in the above reports were done using celloidin sections under the light microscope

The authors of this paper were able to obtain a pair of temporal bones from a case of bilateral sudden deafness which occurred 1 year before the patient s death The right ear is now being processed for study using the conventional celloidin method The left ear was processed for scanning electron microscopy (SEM) Scanning electron micrographs disclosed inner ear pathologies typically f cases of known viral labyrinthitis as will described below The SEM method . to obtain a three dimensional view of changes and to visualize more highly . nified pictures than a light microscope " be able to produce

CASE HISTORY

A 56-year old female complained of sl fever, swelling and congestion of the skin the root of her nose, since April 1976 On morning of September 13, 1976 she not loud tinnitus in her left ear and loss soon afterwards On the next day experienced the same hearing disorders in right ear and became totally deaf on sides The patient did not have acute respira tory infections at that time She felt unsteady when walking after these episodes As she recurrent swelling redness and pain in auricles since October, she was admitted to university hospital in December 1976

Upon admission she showed a saddle deformity, deformed auricles with redness pain on both sides and congestion of epiglottis The following laboratory data we noted RBC, 306×10^4, WBC, 20 100 per mm^3



I Surface view of the cochlear apical turn Encapsu- d tectorial membrane is lying for the most part on the r sulcus (*arrows*) In the basal part the tectorial nbrane is incorporated onto the organ of Corti (*C*) No outer hair cells are visible The area in the box is shown in Fig 2 under higher magnification *R* Reissner's membrane *L* limbus spiralis

R 1° 150 mm, CRP, 5+, RA, +, Wa-R, −, d serum protein, 7 6 g/dl, A/G, 0 64, Ig–A,) mg/dl, Ig–G 2800 mg/dl

Histological study of biopsied auricular tilage showed degeneration of cartilaginous sue and marked infiltration of inflammatory ls and was diagnosed as chondritis No ms of ear disease were observed at otoopy Audiometry showed complete heargloss in both ears Caloric test with 20 ml ice ter irrigation did not elicit any responses spite of administration of steroids, im unosuppressant (azathioprine) and anti otics, the patient took a gradual downward urse and died of gastrointestinal hemorrhage t October, 1977 During the entire course of e illness, no treatment with aminoglycoside tibiotics or irradiation therapy was used utopsy was done 3 hours after death

METHODS

oth temporal bones were removed during au psy and the left ear was immediately pre ared for SEM examination by introducing 2% glutaraldehyde in 1/15 M Sorensen buffer (pH 7 4) several times through the round and oval windows The bone was further fixed in the same fixative for about 24 hours and was subsequently kept in the phosphate buffer

After drilling down the bony capsule the inner ear was dissected in the buffer The cochlea was cut into half turn lengths along the plane running from the modiolus to the border between the oval and round windows Dissected sections were then treated with Murakami s electron conductive fixation method using 2% tannic acid and 1% OsO_4 (Murakami, 1974) Specimens were dehydrated in graded ethanols transferred into amyl acetate and dried in a critical point dryer using liquid carbon dioxide Dried specimens were glued on a brass stub, coated with gold/palladium by an ion sputter and observed under a scanning electron microscope (JSM35)

After observation by SEM, some selected parts of the same specimen were dissected and embedded in Epon One micron sections were then cut using a glass knife and these were observed under a light microscope



Fig 2 Encapsulated and incorporated tectonal membrane (apical turn) Looking through a break in the encapsulating thin cell layer the tectonal membrane is seen to its fibrillar structure (*T*) Degenerated inner hair cells (*arrow*) and distorted inner pillar cells (*P*) are visible *S* inner sulcus cell

FINDINGS

Middle ear structures were not preserved in the specimens because they were removed during autopsy The otic capsule seemed to be more sclerotic than in usual specimens During inner ear dissection no fibrous or bony tissue proliferations or hemorrhage were observed Also no anomalies were found in the cochlea or vestibule As specimens were prepared so as to expose the organ of Corti to the best possible advantage the spiral ligament was partly cut and removed and Reissner s membrane was cut leaving only a small part at the insertion to the spiral limbus It was not ascertained whether the degree of expansion of Reissner s membrane or of the membranous wall of the vestibule was normal or not

Cochlea

The most noticeable pathological changes in the cochlea were a rolled up and encapsulated tectonal membrane and a marked degenera tion of the organ of Corti

The tectonal membrane was visible in a turns except most of the upper basal turn an the beginning of the lower basal turn For th most part the tectonal membrane was lying o the inner sulcus and at places on the organ o Corti Reissner s membrane and on the spira limbus (Fig 1) The tectonal membrane itsel was completely covered by a layer of thin fla cells This covering layer was composed o different types of cells corresponding to the site upon which they rested Where the tector ial membrane was lying on the organ of Corti or the inner sulcus the covering cells had large polygonal surfaces (Fig 2) When the tectorial membrane rested on Reissner s membrane the covering cells were smaller round and had many microvilli (Fig 3B) The encapsu lated tectonal membrane still seemed to keep its fibrillar structure as was observed through





Fig 3 Adhesion of encapsulated tectonal membrane to Reissner's membrane (A B) and destruction of the organ of Corti after ripping off incorporated tectonal membrane (C) The covering cells have round shapes and many microvilli in B (lower basal turn) In (C) the tectonal membrane was partially incorporated on the organ of Corti (C) Only inner hair cells are visible (*arrow*) *R* Reissner's membrane *L* limbus spiralis

small breaks in the monolayer cell cover (Fig 2)

Both the thin cell covering and the tectonal membrane itself were adhered to and incorporated into whichever underlying structure they rested upon (Figs 1 2) for when parts were detached during preparation the underlying part showed a breakdown in structure as if the tectonal membrane had been ripped off (Fig 3C)

In normal cases the surface of the limbus spiralis is usually covered by a thin layer of the tectonal membrane and its cellular structure is hardly visible In this case however the surface was not covered at all by the tectonal membrane Being hidden by round globular projections a cellular structure with polygonal surfaces was visible probably being interdental cells (Fig 4A)

Degeneration of the organ of Corti was marked in all turns being most drastic towards the basal turn All the outer hair cells had disappeared and in turn were replaced by a proliferation of surrounding cells Surfaces of the inner pillar cells were recognizable only in the apical turn In lower turns the inner pillar cells were replaced by polygonal surfaces of what are probably the changed shape of Deiter s cells In the lower basal turn the mound structure of the organ of Corti had degenerated in places into totally flat parts In the cut sections Corti s tunnel and Nuel s space were hardly recognizable in all turns because of proliferation of cellular structure (Fig 4B C)

Inner hair cells with degenerated sensory hairs were seen in every turn except at the basal end of the cochlear duct where they had totally disappeared The sensory hairs lost their normal arrangement and regular stepwise gradation by height (Fig 5) Abno

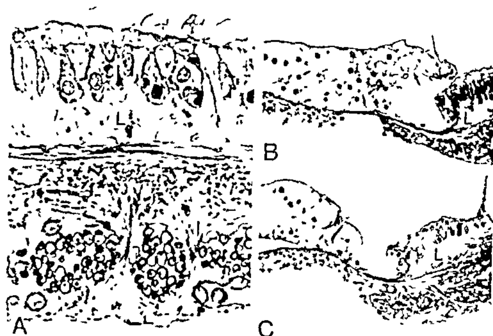
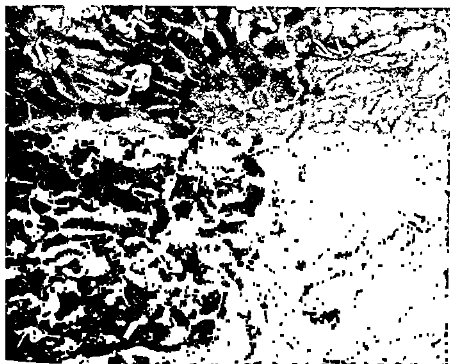


Fig 4 Cut sections of the spiral limbus and the organ of Corti (A) Surface of the spiral limbus (*L*) is covered by interdental cells Myelinated nerve fibers are fairly well preserved (upper basal turn) (B) The tectorial membrane is incorporated into the organ of Corti (*arrow*) turn) Both the tunnel of Corti and Nuel's ... cupied by proliferation of cellular structure in B and (lower middle turn)



Fig 5 Degenerated inner hair cells (apical turn) Regular stepwise arrangement of sensory hairs is lost Large hair-like protrusions are abundant



6 Atrophied saccular macula Sensory cells marked ·creased in number and are replaced by flat cells with ; surfaces

ly large hair-like projections were frently observed

During fixation of the specimen with OsO_4 osseous spiral lamina was examined under ight microscope Here the network of elinated fibers seemed to be fairly well pre-ved except in the basal turn A slight de-ase in number and patchy degeneration e seen in the lower basal turn Myelinated ve fibers were further ascertained in the cut tions of the same specimens (Fig 4 A)

The spiral ligament showed a normal thick-ng and texture The stria vascularis was cut during preparation and was not preserved the observed specimens Reissner's mem-ne also showed its usual appearance, ept for the lower middle and lower basal n where the tectorial membrane had fallen o and adhered to it

stibule

ring dissection of the specimen, a decrease number of otoconia was seen in the utricle and the saccule The gelatinous layer of the otolithic membrane also seemed to be thinner than usual

Observation of the saccular macula showed a great decrease in the number of sensory hair bundles over the entire surface (Fig 6) The remaining sensory hair bundles showed a decrease in the number of sensory hairs and the hairs themselves showed pronounced abnormalities in arrangement and shape (Fig 7) The decrease in the number of sensory cells was not so marked on the utricular macula as in the saccule The central part of the utricular macula, however, was entirely devoid of sensory hair bundles and in their place were found flat cells with large surfaces While all the sensory hair bundles on the saccular macula were degenerated, on the utricular macula many normal looking hair bundles still remained

The ampullary cristae of the anterior ... lateral semicircular canals showed a ...plete loss of sensory hair bundles both ...



Fig 7 D
Regi

central and peripheral parts (Fig 8) The posterior canal crista was not examined as it had been damaged during dissection Prior observation in every vestibular endorgan under a dissection microscope showed that the myelinated nerve fibers were fairly well preserved

COMMENTS

The pathological manifestations which we observed—i e marked atrophy of the organ of Corti encapsulated and dislocated tectorial membrane with no noticeable decrease in the myelinated nerve fibers—are all recognized as histological characteristics of viral labyrinthitis (Lindsay 1967) This same sort of encapsulated tectorial membrane has also been reported in genetically determined deafness (Gussen 1968) and also in congenital deafness of unknown origin (Cohn et a 1968) For our patient there was no fami history of deafness thus sudden deafness this case may well be the result of vir labyrinthitis

Viral labyrinthitis and blood circulation di orders have been postulated as the two mo probable causes of sudden deafness in whic the etiology is not so obvious (Schuknecht al 1973) Our findings give further support favor of the theory of viral labyrinthitis a cause of sudden deafness

The exact cause of the relapsing pol chondritis has not yet been determined Th disease is known not infrequently to a company sensorineural deafness It has bee suggested that vascular impairments (presum ably arteritis of the internal auditory arter are the causative factors of this specific

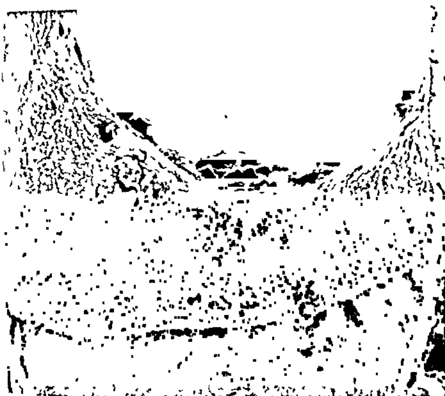


Fig 8 Lateral crista ampullaris viewed from the utricular side The surface of the sensory cell area is completely devoid of sensory hair bundles The surface is covered by flat cells and many globular protrusions

ess and vestibular disorders (Cody et al., 1971) Among 40 relapsing polychondritis cases reported by Cody et al (1971), profound deafness in 4 cases progressed rapidly within hours Medical histories of these suddenly occurred deafness cases showed a great resemblance to the case we have reported in this paper The authors are of the opinion that of the rapidly progressing deafness cases ng during the course of relapsing hondritis might be caused by viral rinthitis However, many more pathological studies on the temporal bone in relapsing chondritis cases are necessary for further cation of the etiology of this deafness.

Using a transmission electron microscope (TEM) Nadol (1977) observed the inner ear in a case of profound deafness which was probably caused by viral labyrinthitis. An encapsulated tectorial membrane was found in parts of cochlear duct Because he noticed a fusion of the tectorial membrane and the connective tissue inside the spiral limbus through a dehiscence of the epithelial layer of the vestibular lip, Nadol mentioned the possibility that the encapsulated substance might be prolapsed connective tissue of the limbus spiralis. In our own case, the encapsulated tectorial membrane was found even on Reissner's membrane without any connection to the limbus spiralis The tectorial membrane observed through breaks in the covering cell layer showed the characteristic fibrillar structure usual to the tectorial membrane These findings seem not to agree with Nadol's assumption, as mentioned

Because of morphological similarities the origin of the layer of cells covering the tectorial membrane seemed to be either the cells of the inner sulcus or the cells of the epithelial layer of Reissner's membrane, depending where the tectorial membrane rested.

labyrinthitis occurred, the tectorial membrane was probably detached from the limbus spiralis Resultant damage to the epithelial cells left marked degeneration of the sensory cells on one side and, on the other hand, caused proliferation of inner sulcus cells and cells of Reissner's membrane through the breakdown of tight junctions These proliferated cells then covered the overlying detached tectorial membrane and then later incorporated themselves with the underlying tissue in the healing stage

The vestibular degeneration which we observed is a rather unique feature according to the findings reported so far on known viral labyrinthitis Degenerative changes in the vestibule in viral labyrinthitis are reported to be mild or confined only to the saccule, showing Scheibe type cochleo-saccular degeneration Vestibular organs of the sudden deafness cases reported by Beal et al (1967) and Schuknecht et al (1973) also showed findings milar to those in cases of viral labyrinthitis As far as can be found from the reports, temporal bones in measles cases (Lindsay et al, 1954, Schuknecht, 1974) were the only ones which showed mild degeneration in the pars superior of the inner ear However, in our case, marked degeneration of the vestibule was found in the cristae ampullares, the saccular macula and utricular macula, in this order of severity The type of virus responsible in the present case could not be ascertained Therefore it is not certain whether the causative virus had affinity to the superior portion of the inner ear or whether the destructive process was more marked than the other cases so far reported

Scanning electron microscopy as applied in this study seems to be one of the most promising methods in human temporal bone histopathology The lower magnifications enable one to survey the overall structure of the cochlear turns Higher magnifications enable one to examine changes at cell level The parts of the inner ear which were examined do not seem to be largely affected by post mortem autolysis After observation by SEM the same specimen can be cut by embedding it either in Epon or in paraffin and then further examined by light microscope or TEM The stria vascularis which regretfully had to be omitted in the present investigation, can be examined in the cut specimen by light microscope and TEM if the tissues containing the stria vascularis are preserved during processing Nerve fibres in the osseous spiral lamina can be checked either by light microscope during specimen preparation or after SEM observation by light microscope or TEM The drawbacks of the SEM method are difficulty in assessing the position of Reissner's membrane and analysing the spiral ganglion cells When examining a pair of temporal bones in which the same pathologies are expected, the authors recommend processing one ear for SEM and the other ear for the conventional celloidin method

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ZUSAMMENFASSUNG

Das linke Ohr einer 57 jahrigen Kranken mit Relapsing Polychondritis die den plotzlichen Horsturz bekam [illegible]

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THE EFFECTS OF EXPERIMENTALLY PRODUCED MIDDLE EAR LESIONS ON TYMPANOMETRY IN CATS

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Abstract Tympanometry was performed before and after producing specific lesions in the middle ears of cats The lesions selected for study included stapes fixation ossicular discontinuity and scarred tympanic membranes Stapes fixation resulted in marked increases in middle ear impedance easily detected with tympanometry Ossicular discontinuity resulted in complex tympanometric shapes which were easily accounted for by simple interactions between acoustic resistance and reactance The complex shapes that occurred in normal and abnormal rs with pressure changing from negative to positive re d from more complicated interactions Large surgical sions in the posterior superior quadrant of the drum were quite visible at otoscopy but could not be detected tympanometrically one month after surgery

Since Terkildsen & Thomsen (1959) originally described the technique, tympanometry has become a widely accepted clinical procedure for the detection of middle ear abnormalities Their original procedure involved measurements of the effects of ear canal air pressure changes on the sound pressure level (SPL) of a tone introduced into the auditory meatus These SPL changes reflect changes in the acoustic input impedance of the middle ear resulting from ear canal pressures that are either positive or negative with respect to atmosphere The technique has been modified by many investigators so that the term tympanometry now refers to a class of observations in which any of a variety of acoustic quantities is measured as a function of ear canal pressure Consequently, the resulting plot (tympanogram) can take a variety of forms depending on the quantity selected for study

Regardless of which acoustic quantity is measured normal tympanograms usually take an inverted V' shape indicating maximum admittance (or minimum impedance) near ambient ear canal pressure Abnormal tympanograms can be grossly categorized as reflecting abnormally high impedance systems or abnormally low impedance systems High impedance systems most commonly result from such pathologies as serous otitis media and ossicular fixation These conditions often manifest themselves in a flattening of the normally peaked tympanogram Although Zwislocki (1957) reported substantial differences in middle ear impedance between normal and otosclerotic subjects Jerger et al (1974) reported that one cannot readily differentiate the otosclerotic tympanogram from the normal tympanogram (p 166) This apparent discrepancy can perhaps be resolved by studying the tympanometric effects of experimentally produced stapes fixation in cats

Low impedance systems are characterized by tympanograms with very high peaks and/or multiple peaks A recent report by Vanhuyse et al (1975) suggested an analytic approach which has revealed some relationships that ac

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count for multiple-peaked tympanograms They postulated that interactions among three quantities—acoustic resistance, acoustic reactance and ear canal air pressure—might account for the multiple-peaked tympanograms that have been observed in normal ears (Alberti & Jerger, 1974, Colletti, 1975, 1976), ossicular disruption (Liden, Harford, and Hallen 1974), and eardrum abnormalities (Feldman 1974) Liden et al (1977) and Margolis & Popelka (1977) studied tympanograms from normal and pathological human ears and basically confirmed the rules outlined by Vanhuyse et al that govern the occurrence of double peaked tympanograms These rules are most clearly described by the graphic presentation provided by Vanhuyse et al and may be summarized as follows Double-peaked tympanograms occur when 1) acoustic reactance is stiffness-controlled and less than acoustic resistance near ambient ear canal pressure and greater than acoustic resistance at high pressures, and 2) when acoustic reactance is mass-controlled near ambient pressures and stiffness controlled at high pressures When double-peaks occur in acoustic conductance, susceptance and impedance tympanograms, the acoustic resistance and reactance tympanograms usually have much simpler shapes

Margolis & Popelka (1977) suggested that double peaked tympanograms at 660 Hz may occur in both normal and pathological ears Double peaked tympanograms from stiffness-controlled ears can be accounted for on the basis of normal interactions among tympanometric quantities, while those that occur in mass controlled ears probably result from some past or present pathology The distinction between mass-controlled and stiffness-controlled reactance at 660 Hz may provide a simple method for identifying the double-peaked tympanograms that result from pathology This distinction can easily be made on the basis of the B_a tympanogram If the minimum in the center of the notch is the lowest point on the tympanogram, the ear is mass controlled

Although there is a substantial literature concerned with tympanometric results in pathological ears, the effects of specific middle ear lesions on tympanometry are not well understood This confusion derives in large part from poor control over the nature and extent of the lesions in clinical populations of human subjects We undertook the present experiments to 1) study the effects of specific, experimentally-produced middle ear lesions on tympanometry in cats, and 2) evaluate the Vanhuyse et al predictions concerning the nature of W-shaped tympanograms The lesions selected for study included stapes fixation and ossicular discontinuity (Experiment I) and healed tympanic membrane perforation (Experiment II)

EXPERIMENT I

Methods and Materials

Tympanometric measurements were performed by simultaneously monitoring the acoustic conductance (G_a) and acoustic susceptance (B_a) outputs of an acoustic admittance meter (Grason Stadler 1720B) with a two-channel strip-chart recorder (Gould 220) A third pen was used as a manually-triggered event marker that provided estimates of ear canal air pressure The event marker was triggered by the experimentor in 50 mmH$_2$O intervals during each tympanometric record Since air pressure is a linear function of time with this instrument, reasonable estimates of ear canal pressure can be obtained in this manner For each experimental condition, tympanograms were recorded with two probe frequencies (220 and 660 Hz) with both increasing (−400 to +400 mmH$_2$O) and decreasing ear canal air pressure All data were corrected to the plane of the tympanic membrane by the MAX/MIN procedure (see Margolis & Smith, 1977) Acoustic admittance data (B_a and G_a) are expressed in acoustic mmhos acoustic impedance data (X_a and R_a) are pressed in acoustic ohms One acoustic is defined as m^3×10^5/Pa·sec, one a ohm is defined as μPa·sec/m^3

Table I[a]

		220 Hz					660 Hz				
		G_a	B_a	R_a	$-jX_a$	Z_a	G_a	B_a	R_a	$-jX_a$	Z_a
Normal (A) (before drilling)	$\bar{X}$	0 05	0 49	214	2 147	2 161	0 64	1 47	245	559	625
(N=13)	S D	0 30	0 08	158	359	366	0 31	0 37	132	108	94
Normal (B) (after drilling)	$\bar{X}$	0 06	0 50	223	2 031	2 046	0 49	1 47	205	624	660
(N=8)	S D	0 04	0 10	108	380	380	0 21	0 23	81	170	123
Stapes fixation	$\bar{X}$	0 05	0 25	713	3 929	3 999	0 14	0 83	230	1 213	1 239
(N=6)	S D	0 02	0 03	105	671	649	0 08	0 18	141	780	793
Stapedectomy	$\bar{X}$	0 46	1 87	106	722	734	1 55	1 97	743	301	454
(N=8)	S D	0 51	1 13	63	475	470	1 83	1 31	256	213	700
Incudo-stapedectomy	$\bar{X}$	1 50	1 97	223	568	631	1 58	0 89	375	768	517
(N=6)	S D	1 60	1 18	161	470	465	0 98	1 02	238	750	148

[a] Ga and Ba data are in acoustic mmhos Ra Xa and Za data are in acoustic ohms

Experimental subjects included 13 cats, otoscopically screened for obvious middle ear pathology After the cats were sedated with Nembutal (35 mg/kg intraperitoneal) the pinna and all but the medial 1 0 cm of the ear canal were removed The middle ear was entered through a 2 5 mm burr hole placed 4 mm posterior–superior to the opening of the bony ear With the aid of a binocular microscope following middle ear manipulations were First the stapes was immobilized with methyl methacrylate glue (Eastman 810) Then the stapes was removed Finally, the incus was removed

Tympanograms were recorded after each of these manipulations with the burr hole sealed with a plug made from dental cement Experimental conditions included the following There were two baseline conditions, one before and one after drilling into the middle ear This allowed the determination of the potential traumatic effect of drilling on tympanometric results There were three experimental conditions representing each of the experimentally-produced lesions stapes fixation, stapedectomy, and incudostapedectomy Tympanograms, recorded in acoustic conductance G_a, and susceptance, B_a, were converted to acoustic resistance, R_a, reactance X_a and impedance, Z_a, by the relations derived by Van Camp (1977) and Margolis & Popelka (1977) Although measurements were made from 13 cats, surgical complications required the elimination of several animals from the experiment The most common problems were tympanic membrane rupture and excessive glue in the middle ear Agreement between tympanograms recorded before and after drilling was required for inclusion of the experimental data Table I presents the pre and post-drilling results (Baseline A and B) indicating excellent agreement for those subjects retained in the experiment

Results and Discussion

Direction of pressure change

Fig 1 presents representative results from one cat illustrating the effects of direction of pressure change Similar relations were evident in the data from each animal These tympanograms were recorded with a 660 Hz probe before drilling into the middle ear In the decreasing (positive to negative pressure direction) G_a and B_a tympanograms assume the normal, single peaked tympanometric shape R_a and X_a show the relations predicted by Vanhuyse et al (1975) and confirmed in human subjects by Liden et al (1977) and Margolis & Popelka (1977) Specifically, acoustic reactance is greater (in absolute value) than resistance for all ear canal pressures and resistance assumes a grossly asymmetrical shape In the increasing pressure direction G_a and B_a tympanograms are substantially differ

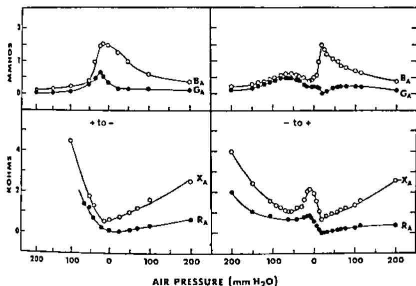


Fig 1 The effects of direction of pressure change on tympanograms from one cat The data in the left panels were recorded with pressure changing from positive to negative with respect to atmosphere The data on the right were recorded in the opposite direction The top panels present acoustic susceptance (B_a) and conductance (G_a) The bottom panels are acoustic reactance ($-X_a$) and resistance (R_a) computed from the B_a and G_a data All data are corrected to the plane of the tympanic membrane by the MAX/MIN procedure (see Margolis & Smith 1977)

ent At about 20 mmH_2O acoustic conductance is very near zero Acoustic susceptance has a double peak with the larger peak corresponding to the minimum in the G_a tympanogram The resistance and reactance tympanograms take much more complicated forms than occurred with the other pressure direction Both are asymmetrical and double-peaked Several investigators have reported differences in tympanograms corresponding to different pressure directions The position of the tympanometric peak was found to be dependent upon pressure direction by Woodford et al (197 , & Feld & Wins and Williams & Feldman (1976) found a higher occurrence of double peaked tympanograms when pressure changed from negative to positive than for the reverse direction These findings are similar to the present study However the double peaked B_a tympanogram in Fig 1 appears to be unrelated to those predicted by Vanhuyse et al (1975) and reported by Liden et al (1977) and Margolis & Popelka (1977) In those reports double peaked G_a and B_a tympanograms never resulted from double peaked R_a and X_a tympanograms like those shown in Fig 1 Rather, much simpler tympanometric patterns in R_a and X_a resulted It appears then, that the double peaked tympanograms that occur with the increasing pressure direction result from a different kind of interaction between resistance and reactance than those that were predicted by Vanhuyse et al In the following discussion of the effects of middle ear lesions, only tympanograms recorded with the decreasing (positive to negative) direction will be considered

Normal ears

Figs 2 and 3 present acoustic conductance and susceptance tympanograms from one resentative animal for two experimental tions—stapes fixation and ossicular tinuity Acoustic resistance and ta computed from the measured

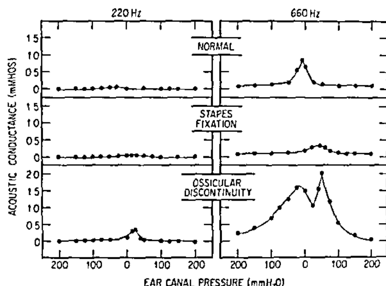


Fig 2 Acoustic conductance tym nograms from one cat at two probe f quencies (220 and 660 Hz) under three perimental cond tions Normal after d ling into the bulla with the drill hole seal stapes fixation after cementing the sta footplate ossicular discontinuity after moval of the stapes All data are correc to the plane of the tympanic membrane

and susceptance values are presented in Figs 4 and 5 for two probe frequencies, 220 and 660 Hz, and two experimental conditions—the normal control condition and ossicular discontinuity Averaged data for all conditions, probe frequencies, and acoustic quantities are mmarized in Table I

Tympanograms recorded from normal cats similar to those obtained with human subjects Conductance and susceptance tympanograms (Figs 2 and 3) have single peaks located near ambient ear canal pressu with the exception of G_a at 220 Hz whi similar to human infant data (Margolis Popelka, 1975), does not have a clearly defin peak The relations between acoustic sistance and reactance (Figs 4 and 5) are si lar to those described for normal human s jects (Vanhuyse et al 1975, Liden et al 19 Margolis & Popelka, 1977) Specifical acoustic reactance is stiffness controlled greater (in absolute value) than acoustic

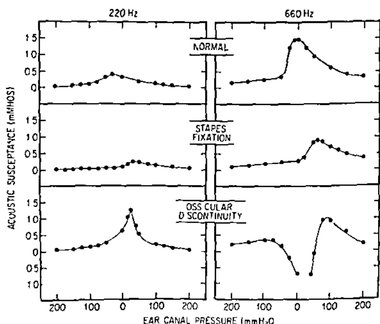


Fig 3 Acoustic susceptance tym nograms from one cat See caption to F

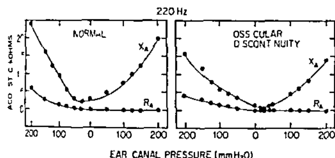


Fig 4 Acoustic resistance (R_a) and reactance ($-X_a$) tympanograms computed from the 220 Hz data in Figs 2 and 3 for the normal condition and after removal of the stapes

sistance for all ear canal pressures at both probe frequencies Furthermore, acoustic reactance is nearly symmetrical about ambient pressure while resistance takes a grossly asymmetrical shape with lower values for positive than for negative pressures These relations are associated with single-peaked B_a and G_a tympanograms in humans and, as these data suggest in cats as well Static acoustic impedance (see Table I and Fig 6) is somewhat higher than for adult human subjects (Margolis & Smith, 1977) but similar to 2–4 month human infants (Margolis & Popelka 1975)

Stapes Fixation

Figs 2 and 3 present tympanometric results from one cat illustrating the effects of stapes fixation on acoustic susceptance and conductance tympanograms In all cases there was a marked decrease in the height of the tympanometric peak except for the 220 Hz G_a tympanogram which in normal animals did not have a clearly defined peak These changes were associated with increases in both acoustic resistance and acoustic reactance, although at 660 Hz, the resistive change was small (Table I) As Zwislocki (1957) pointed out, stapes fixation produces a marked increase in middle ear impedance The Jerger et al (1974) contention that normal subjects and otosclerotics cannot be differentiated on the basis of tympanometry may be related to the following considerations First, the effects of otosclerosis on the input impedance of the human middle ear may be fundamentally different from the effects of our experimentally-produced stapes fixation in cats Second, normal variability among human subjects is probably greater than among our carefully selected animal subjects, making departures from normality more difficult to detect in humans Third, the quantification of tympanometric data in arb[illegible] units and the classification of tympanograms into a few poorly defined categories may obscure potential differences that could be detected by more rigorous measurement procedures On visual observation the tympanograms shown in Figs 2 and 3 for the stapes fixation condition are not clearly different from normal However, when the data are converted to estimate the static

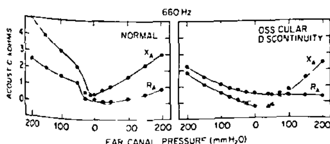


Fig 5 Acoustic resistance (R_a) and reactance ($-X_a$) tympanograms compute from the 660 Hz data in Figs 2 and 3 for t normal condition and after removal stapes

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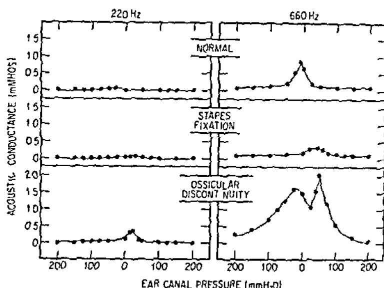


Fig 2 Acoustic conductance tympanograms from one cat at two probe frequencies (220 and 660 Hz) under three experimental conditions Normal after drilling into the bulla with the drill hole sealed, *stapes fixation* after cementing the stapes footplate, ossicular discontinuity after removal of the stapes All data are corrected to the plane of the tympanic membrane

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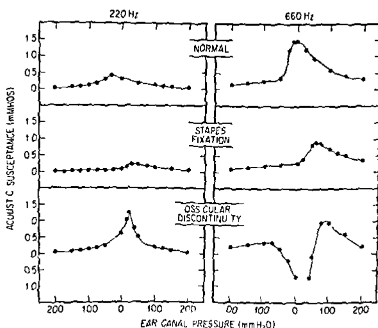


Fig 3 Acoustic susceptance tympanograms from one cat See caption to Fig 2

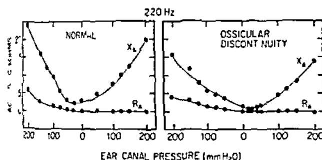


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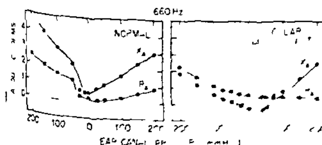


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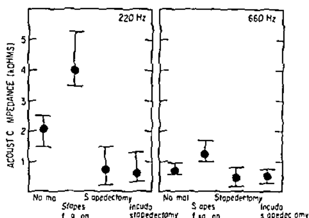


Fig 6 Mean and range static acoustic impedance (corrected to the plane of the tympanic membrane) for all experimental conditions at two probe frequencies (220 and 660 Hz)

acoustic impedance of the middle ear, the two groups—normal and stapes fixation—separate into two distinctly different distributions (see Fig 6)

Ossicular discontinuity

Representative results from one animal after removal of the stapes are illustrated in Figs 2–5 At 220 Hz, ossicular discontinuity resulted in single-peaked B_a and G_a tympanograms, although when compared with the normal control condition the amplitudes of the peaks were increased (Figs 2 and 3) Fig 4 illustrates that the relationship between resistance and reactance is similar to the normal condition That is, reactance is stiffness-controlled and always greater, in absolute value, than resistance At 660 Hz, susceptance and conductance tympanograms have double peaks (Figs 2 and 3) In Fig 5 it is evident that the normal relation between resistance and reactance is altered In the stapedectomized subject, reactance is mass controlled near ambient ear canal pressure and stiffness-controlled at high positive and negative pressures The data obtained after removal of the incus were qualitatively similar to the stapedectomy condition The B_a and G_a tympanograms tended to be more dramatically notched and the reactance tympanogram was more displaced toward positive values In general, the results from cats with ossicu discontinuity are similar to the data we ported from a human subject with a s pected discontinuity (Margolis & Popel 1977, figures 9 and 10) Both the human a feline data support the Vanhuyse et al pred tions regarding interactions among R_a and that account for double peaked ty panograms Specifically, double peaked and G_a tympanograms occur when reacta is less than resistance and/or mass control near ambient pressure, and reactance stiffness controlled and greater than resistan at high ear canal pressures

Summary and Conclusion

Mean static acoustic impedance data for experimental conditions are summarized Fig 6 Stapes fixation resulted in a marl increase in acoustic impedance at both pro frequencies Ossicular discontinuity (s pedectomy and incudo-stapedectomy) sulted in a clear decrease in acoustic im dance at 220 Hz At 660 Hz the effect of sicular discontinuity on static impedance v not as dramatic, even though 6 of the stapedectomized ears and all incudo pedectomized ears were mass controll The lack of separation between the norm and the ossicular discontinuity groups (Fig is due to the fact that static impedance val are insensitive to the sign of reactance T is, a mass controlled ear may have the sa static impedance as a normal stiffness-c trolled ear It is necessary, therefore analyse the components of acoustic im dance if high frequency probe tones are to clinically useful

The interactions among resistance a reactance, predicted by Vanhuyse et al (19 to account for multiple peaked B_a and tympanograms have been confirmed in h man subjects by Lidén et al (1977) and M golis & Popelka (1977), and now in w controlled middle ear lesions produced experimental animals Double peaked ty panograms that occur in normal cats wit

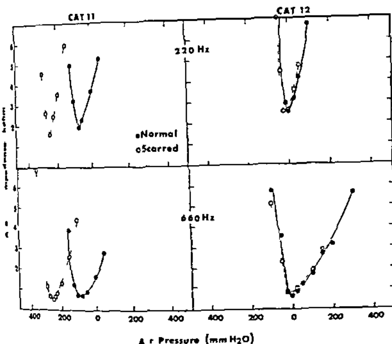


Fig 7 Acoustic impedance tympanograms ($|Z_a|$) from 2 cats at two probe frequencies (220 and 660 Hz). One ear of each cat was perforated one month before these measurements were made. The data are corrected to the plane of the tympanic membrane.

660 Hz probe and with pressure changing from negative to positive are an exception to the Vanhuyse et al predictions. These tympanograms are characterized by complex resistance and reactance patterns that represent a more complicated set of interactions than those that seem to be associated with ossicular disruption and eardrum abnormalities. The effect of pressure direction on tympanometric shape in human subjects requires further study. If effects similar to those depicted in Fig 1 can be demonstrated in human subjects, more attention should be paid to the direction of pressure change in clinical procedures.

EXPERIMENT II

Methods and Material

Tympanograms were recorded from the operated ear and the unoperated control ear of 2 cats 1 month after perforation of the tympanic membrane. In one ear of each cat a 2 [illegible] perforation was produced with a [illegible] knife in the posterior-superior quadrant of the eardrum. This region has been shown to vibrate maximally to acoustic stimulation (Khanna & Tonndorf 1971). Thus we reasoned that if a scar resulting from a surgical incision would influence tympanometry, its effect would be maximal in this location. Measurement procedures were identical with those of Experiment I.

Results and Discussion

Although the scars were easily visible at otoscopy, there was no apparent effect on tympanometric results. Fig 7 presents acoustic impedance tympanograms from both ears of both experimental animals at two probe frequencies. Cat 11 had a substantial amount of negative pressure in the operated ear but Z_a measures were not appreciably different. Cat 12 had virtually identical tympanograms from the two ears.

Conclusion

The data of Experiment II suggest that scars resulting from surgical incision, even when [illegible] large and located in the [illegible] posterior [illegible] of the [illegible], do not cause lasting change in the acoustic impedance [illegible] middle ear [illegible]

tympanometry. Results from human subjects have demonstrated remarkable effects of tympanic membrane abnormalities on tympanometric data (Feldman, 1974; Margolis & Popelka, 1977). These eardrum abnormalities represent gross changes in the mechanoacoustic properties of the eardrum that result from anatomical changes that characterize healed spontaneous perforations. The present data do not contradict the well-documented effects of scarred eardrums, but demonstrate that the healing that occurs after surgical incisions does not leave the eardrum in a condition that is mechanically different from the normal ear.

ZUSAMMENFASSUNG

Tympanometrie wurde vor und nach Erzeugung von spezifischen Schädigungen des Katzenmittelohrs durchgeführt Die für diese Arbeit ausgewählten Schädigungen bestanden aus Steigbügelversteifung, Unterbrechung der Horknochelchen und narbigem Trommelfell Die Steigbügelversteifung verursachte deutliche Erhohungen in der Mittelohrimpedanz, die durch Tympanometrie leicht entdeckt wurde Unterbrechung der Horknochelchen hatte omplizierte tympanometrische Formen zur Folge, die durch einfache Wechselwirkungen zwischen akustischem Widerstand und Reaktanz leicht erklarbar waren Die komplizierten Formen, die in normalen und geschädigten Ohren vorkamen, als der Luftdruch von negativ auf positiv gebracht wurde, ergaben sich von komplizierteren Wechselwirkungen Große chirurgische Einschnitte im hinteren, oberen Quadrant des Trommelfells waren mittels Otoskopie ganz sehbar, konnten aber einen Monat nach dem Einschnitt durch Tympanometrie nicht entdeckt werden

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NASAL CANCER ASSOCIATED WITH OCCUPATIONAL EXPOSURE TO ORGANIC DUST

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Abstract In order to study the association of carcinoma of the nose and paranasal sinuses with occupational exposure to dust the cancer registry of the National Board of Health and Welfare was consulted as information source The material was divided into two groups the first consisting of consecutive cases of adenocarcinoma from 1961 until 1971 and the second of cases of squamous cell and poorly differentiated carcinoma from 1965 until 1971 Th[illegible]

[illegible] gistry of the Swedish Church The predominant occupational group among men with adenocarcinoma was joiners who comprised 19 cases (53%) At least 12 of these had been cabinet makers The period of exposure to wood dust among the joiners was known in 9 cases and was estimated in most of the cases The duration of exposure was not less than 25 years in any case Most joiners had been working with different species of hard wood There was no predominant occupational group among men with squamous cell and poorly differentiated carcinoma although the occupations recorded were in many cases associated with a working environment containing various kinds of organic dust Among the females with squamous cell and poorly differentiated carcinoma there were a number of cases who had occupations associated with organic dust such as textile work leather and flour handling The investigation shows that the bulk of males with adenocarcinoma of the nose and paranasal sinuses had been exposed to wood dust

In 1967 Acheson et al reported a strong association of adenocarcinoma of the nose and paranasal sinuses with occupational exposure to wood dust They found an excess of adenocarcinoma among workers in the furniture industry of High Wycombe, England where the average annual incidence rate was estimated to 0 6 per 1 000 Similar findings have been reported from Belgium (Debois 1969) Denmark (Mosbech & Acheson, 1971 Andersen, 1975), France (Gignoux & Bernard, 1969, Gignoux et al , 1971), Holland (Delemarre & Themans, 1971) and Australia (Ironside & Matthews, 1975) It seems that workers exposed to fine wood dust, for instance furniture makers, are particularly at risk

In order to ascertain to what extent carcinoma of the nose and the paranasal sinuses is associated with dusty occupations in Sweden, the cancer registry of the National Board of Health and Welfare was consulted as information source This registry receives reports on newly detected cases of cancer and related diseases from the whole country The registry material is based upon independent reports from physicians, pathologists and cytologists Reporting to the registry is statutory and in practically all hospitals a matter of routine Reports from clinical pathologists can be regarded as 95–100% complete

MATERIALS AND METHODS

The analysed cases of carcinoma of the nose and the paranasal sinuses are based on registry reports from 1961–70

The material was divided into two groups, the first consisting of cases of adenocarcinoma from 1961 until 1971, and the second of cases of squamous cell and poorly differentiated carcinoma from 1965 until 1971

The first group consisted of 36 men and 10 women, the latter of 127 men and 85 *women* All hospitals and physicians who reported to the registry were contacted and all subje still surviving received a questionnaire

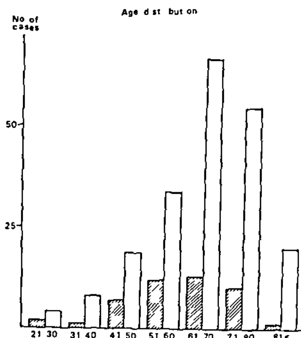


Fig 1 Age distnbution Hatched bars adenocarcinoma open bars squamous cell and und fferentiated carcinoma

was completed in more than 90% of cases The patients stated their present and previous cupation, with special reference to various es of dusty surroundings There were also questions concerning previous and present smoking and snuff taking habits

The same type of questionnaire was also sent to close relatives of the deceased where these could be traced In almost 50% of all recorded cases the questionnaires were an swered In the remainder information on pre vious occupations was received from the per sonal records of the population registry of the Swedish Church

The total case material and age distribution is presented in Fig 1 The mean age of the adenocarcinoma subjects was 62 years and of the squamous cell and poorly differentiated cancer cases 64 years The crude annual in cidence rate is presented in Fig 2 which shows the rate of adenocarcinoma in males and the rate in males of total malignancies of the nose and paranasal sinuses

The geographical distribution of the male adenocarcinoma cases was recorded according to the latest known domicile The cases were rather evenly distributed in central Sweden and there was no apparent clustering Isolated cases only were found in the northern and most southerly parts of Sweden Hardly any cases were found in the big cit es Most cases were localized to small towns and vil lages many of which had furniture industries

RESULTS

The occupational distribution among men with adenocarcinoma is presented in Table I Of 36 cases the questionnaire was answered in 18 As many as 19 men (53%) had evidently been joiners and at least 12 of these had been cabinet makers Among other occupation represented 3 persons had worked in the flour industry

The period of exposure to wood dust among the joiners is established in 9 cases and i shown in Fig 3 The arrows indicate the time of diagnosis In a few other cases the time of exposure is more uncertain with an obviou latency period of 5 to 30 years In all cas the duration of exposure is 25 years or more

Most joiners in this survey had been work ing with different species of hardwood such a oak teak mahogany and birch and no one ha exclusively handled softwood such as spruce or pine

The smoking and snuff taking habits were recorded from the questionnaires only and the pattern was no different from the finding in a general survey There were two snuff takers among the joiners

The occupations of the males with squa mous cell and poorly differentiated carcinoma are recorded in Table II which shows tha questionnaires were returned in 6? cases of 127 As is seen no one occupational group dominates However many of the occupation recorded are associated with a dusty sur rounding and we found a few subjects who had worked with stone drilling textiles leath er and flour The number of cases is too smal to be of any certain significance The joiners ir

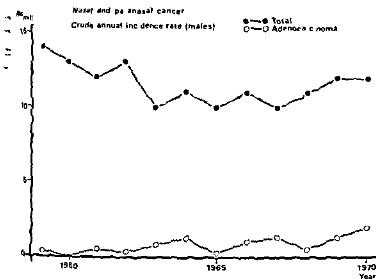


Fig 2 Crude annual incidence rate in males

this group represented only 4% of the total number of cases

Information on the occupations of the females was scarce and it was not possible to trace the occupations of the 10 female cases of adenocarcinoma Table III shows the distribution of occupations among the 85 females with squamous cell and low differentiated carcinoma Only 31 cases answered the questionnaires The occupations with exposure to organic dust such as textile work, leather and flour handling are well represented in this group However, the bulk of the cases either come under the heading 'miscellaneous', which covers housekeeping, secretarial work, etc , or else the heading 'unknown'

DISCUSSION

Following the reports from England and other countries, we felt a need to investigate whether there is an association of cancer of the nose and paranasal sinuses with exposure to wood dust in Sweden At the same time we could seek other types of occupation with environmental dust possibly associated with cancer of the nose

Table I *Occupation of males with adenocarcinoma 1961–70*

Occupation	No	Percentage	Questionnaire +
Joiners	19	53	10
Building workers	1	3	–
Fitters	6	17	4
Transport workers	3	8	1
Flour workers	3	8	2
Miscellaneous	4	11	1
Total	36	100	18

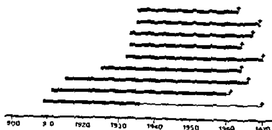


Fig 3 Time of exposure to wood dust and nine woodworkers

Table II *Occupation of males with squamous cell and poorly differentiated carcinoma 1965–70*

Occupation	No	Per centage	Question naire+
Joiners	5	4	2
Other woodworkers	8	6	5
Building workers	10	8	6
Fitters	23	18	12
Farm labourers	23	18	12
Stone drillers	3	2	3
Transport workers	6	5	6
Textile workers	6	5	2
Flour workers	1	1	–
Leather workers	1	1	–
Miscellaneous	25	20	14
Unknown	16	13	–
Total	127	101	62

The cancer registry of the National Board of Health and Welfare in Sweden is well established and is intended to cover at least 95% of all new cancer cases in Sweden A possible way to study the problem was to analyse a case material from the registry res We could not, however, obtain any information from the registry regarding the domicile, occupation and survival of the patients There is a delay of about 5 years from the time of diagnosis until processed information from all reported cancer cases is available at the cancer registry, being the reason why only cases diagnosed before 1971 were analysed

It has been discussed whether it is in accordance with ethical practice to make direct contact with patients whose names and identification have been extracted from a computerized cancer registry We therefore informed all physicians who had been in charge of the patients about the project and asked for permission to contact their patients In fact two cases had to be withdrawn from the study due to a refusal from one doctor Through the physicians, the hospitals and the population registry of the Swedish Church we obtained information about the domicile of the patients still alive, and on dates of death Furthermore, we discovered that the population registry had personal records where the occupation of the deceased was noted during his adult life Unsuccessful attempts were made to trace the occupations of the patients through the Swedish Census The questionnaires distributed were generally appreciated and we did not experience any negative attitude to them

In accordance with previous reports and judging from our preliminary investigation it seemed relevant to separate the cases of adenocarcinoma from the cases of squamous cell and poorly differentiated carcinoma The case material shows a male/female ratio of 3 6 1 for adenocarcinoma and 14 1 for squamous cell and poorly differentiated carcinoma The crude annual incidence rate of males (Fig 2) shows no secular trend, especially regarding adenocarcinoma The geographical distribution of male cases of adenocarcinoma does not show any clustering which may be due to the fact that there are a number of small furniture firms scattered over a large part of Sweden Another cause may be that some of the males were retired at the time of the diagnosis and had moved from the area in which they had been working

The occupational group of males with adenocarcinoma (Table I) is dominated by joiners who made up 53% of all male cases This number demonstrates convincingly that there is an association between adenocarcinoma in males and the joinery occupation According

Table III *Occupation of females with squamous cell and poorly differentiated carcinoma 1965–70*

Occupation	No	Per centage	Question naire+
Textile workers	8	9	6
Leather workers	3	4	3
Farm labourers	6	7	2
Flour workers	3	4	2
Miscellaneous	48	56	18
Unknown	17	20	–
Total	85	101	31

to previous reports, it is reasonable to suggest that this association depends on the exposure to wood dust From the questionnaires received we could not draw any conclusions regarding the type of wood involved Most joiners had worked with different species of wood including hardwoods One case only had worked almost exclusively with birch and pine The question remains open, whether dust from softwoods such as pine and spruce might be associated with carcinoma

It appears that the exposure period to the dust must as a rule cover several years, which is in accord with the findings of other investigators (Acheson et al , 1968, Andersen, 1975, Gignoux et al , 1971) The period from the last exposure to the time of the diagnosis appeared to vary from 0 to 30 years or more There was no indication that smoking or snuff taking habits had any influence on the development of carcinoma of this type

In order to calculate the relative risk for joiners we have tried to obtain adequate figures on the number of males actually occupied with this kind of work The Wood Workers Union in Sweden has an estimation of 25 000 working in the wood industry, with exposure to wood dust This number has not changed significantly during the last 40 years At the 1960 Swedish Census, the number of males occupied in the furniture industry was 17 000 These figures give us a relative risk of 165 and 109 for joiners and furniture workers respectively as a raw estimate In calculating this it is assumed, naturally that the reference population consisting of males otherwise occupied is not exposed to wood dust and that other factors giving rise to a risk for this tumour are evenly distributed in the population We also disregarded persons not traced and—in so doing—assumed the proportion of cases to be identical among the non traced Furthermore it is assumed that the populations compared are constant during the period of time covered by the study Thus, a raw estimate is based on a series of assumptions some of [illegible] ie

The calculated risk ratio is therefore given only to demonstrate the order of risk magnitude Due to the uncertain and probably overestimated numbers the relative risk may accordingly be considerably higher than calculated

Among the occupations represented by the males with adenocarcinoma, flour workers should be noticed, but their number is too small to be of any certain significance The duration of exposure in these cases varied extensively

The present investigation of the occupation of cases with squamous cell and poorly differentiated carcinoma of the nose and paranasal sinuses was carried out in order to find out whether joiners or other workers exposed to dust were at risk (Table II) Although there is no occupational group dominating the material, we found a great number of workers with occupations where exposure to organic dust is common However, it is not possible to draw any conclusion as to a direct association with cancer There were only 5 joiners and 8 other woodworkers among 127 male cases, which should be compared with the large number of joiners with adenocarcinoma

It appeared difficult to obtain information on previous occupation from women with nasal carcinoma There were 10 cases of female adenocarcinoma, one had been a textile worker, 2 had been part time farm labourers and the remaining 7 had no definite occupation recorded Women with squamous cell and poorly differentiated carcinoma (Table III) had been working mainly in environments without known exposure to dust but there was a small number of women whose occupations had been connected with textiles, leather and flour

The present investigation shows that woodworkers in Sweden who have been exposed to wood dust for a long period run a greater risk of developing adenocarcinoma of the nose and paranasal sinuses than other [illegible] the population, which is a [illegible] the reports from England and other

ACKNOWLEDGEMENT

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ZUSAMMENFASSUNG

Beim Studium des Zusammenhanges von Karzinom der Nase und Nebenhohlen mit berufsmaßiger Exponierung von Staub diente das Cancerregister der schwedischen Sozialverwaltung als Informationsquelle Das Material wurde in zwei Gruppen eingeteilt Die erste Gruppe umfaßt samtliche Fälle von Adenokarzinom 1961 bis 1971, die zweite Gruppe sämtliche Falle von Plattenepithel- und undifferenzierten Karzinomen *1965* bis *1971* In der ersten Gruppe waren 36 Manner und 10 Frauen, in der zweiten 127 Manner und 85 Frauen Die Information über ausgeübte Berufe war durch Fragebogen und aus dem Personenstandregister ersichtbar Die vorherrschende Berufsgruppe der Manner mit Adenokarzinomen waren Tischler (19 Falle, 53%) Wenigstens 12 von *diesen waren Mobeltischler Die Exponierungszeit fur* Holzstaub bei Tischlern war in 9 Fallen bekannt und in den ubrigen Fallen geschatzt Bei jedem der Patienten war die Exponierungszeit nicht weniger als 25 Jahre Die Tischler arbeiteten mit mehreren Holzsorten Keine Berufsgruppe der Manner mit Plattenepithel- und undifferenziertem Karzinom war dominierend, auch wenn mehreren Berufen verschiedene Sorten organischen [illegible]bes im Arbeitsmilieu vorkamen Bei einigen Frauen [illegible] Plattenepithel- und undifferenziertem Karzinom [illegible]urden Berufe mit organischem Staub, wie bei Textil-, Leder und Getreidearbeit, vorgefunden Die Untersuchung zeigt, daß die Mehrzahl der Manner mit Adenokarzinom in Nase und Nebenhöhlen im Berufsleben dem Holzstaub ausgesetzt waren

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OCCURRENCE AND DISTRIBUTION OF VIP NERVES IN THE NASAL MUCOSA AND TRACHEOBRONCHIAL WALL

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bstract Nerves displaying vasoactive intestinal peptide IP) immunoreactivity were detected in the upper res iratory tract of guinea pigs rabbits and cats VIP nerves ere numerous in the cat less numerous in the rabbit and re in the guinea pig In the nasal mucosa fine varicose IP nerves were found to surround nasal glands and small ood vessels In the tracheobronchial wall VIP nerves ere observed around seromucous glands blood vessels d smooth muscle Ganglia located in the walls of the achea and main bronchi contained clusters of VIP im unoreactive nerve cell bodies conceivably representing e origin of the VIP fibres found in this region

soactive intestinal peptide (VIP) isolated Said & Mutt in 1970 has been generally ught of as a hormone (Grossman, 1974) wever, recent immunohistochemical stud have revealed that VIP occurs in neurons P nerves are numerous in the oesophagus dman et al, 1978*a*) gastro-intestinal tract yant et al, 1976, Larsson et al, 1976, dler et al, 1977, 1978) and genito uri y tract (Alm et al, 1977 Larsson et al, 7*a b*)

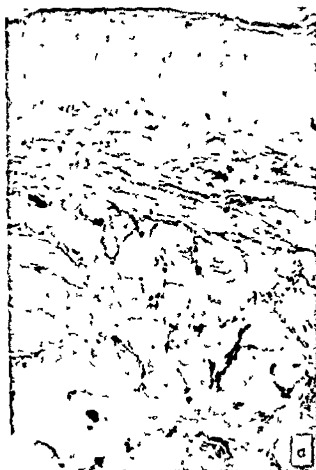
3oth the nasal mucosa and the tracheo nchial wall are richly supplied with adren gic nerves (Dahlstrom & Fuxe, 1965, Dahl om et al, 1966 Anggård & Densert, 1974) ere is also a rich supply of acetylcholines ase (AChE) positive nerves in both these ations (Ishii 1970 Mann 1971) In addi n electrophysiological and pharmacological idies indicate the presence of non cholin gic, non adrenergic nerves in the respiratory ct (cf Burnstock 1975)

The present report describes the occurrence and distribution pattern of VIP nerves in the upper respiratory tract, with emphasis on the nasal mucosa and tracheobronchial wall

MATERIAL AND METHODS

Adult guinea pigs, rabbits and cats were used, 5 of each species Guinea pigs were killed by exsanguination under diethyl ether anesthesia Rabbits were killed by an overdose of pentobarbital sodium, and cats by exsanguination under light pentobarbital sodium anesthesia Specimens were taken from the nasal mucosa, epipharynx, mesopharynx, larynx and from the tracheobronchial tree All specimens were frozen to the temperature of liquid nitrogen in a propane–propylene mixture and freeze dried They were then fixed by exposure to diethylpyrocarbonate vapour at 55° for 3 hrs (Pearse et al, 1974), and embedded in paraffin *in vacuo* Sections were cut at 5 μm and deparaffinized in xylene

For the demonstration of VIP immunoreactivity the sections were subjected to an in direct immunofluorescence method (Coons et al, 1955) or to the peroxidase–antiperoxidase (PAP) procedure (cf Sternberger, 1974) VIP antiserum (code no 5603) was kindly supplied by Drs J Fahrenkrug and O Schaffalitzky de Muckadell, Department of Clinical Chemistry, Bispebjerg Hospital, Copenhagen, D mark The antiserum has been in detail elsewhere (Fahrenkrug & litzky de Muckadell, 1977) It was





1 Cat nasal mucosa maxilloturbinal area Immuno histochemical demonstration of VIP (a) PAP procedure Numerous VIP immunoreactive nerve terminals running close to acini of seromucous glands Surface epithelium at the top of figure (×200) (b) Section through seromucous gland Immunofluorescent VIP nerves among acini as well as around blood vessel (arrows) (×150)

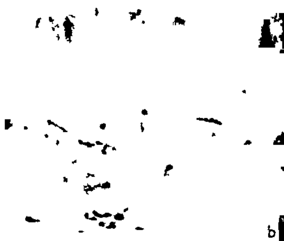
tion 1 80 for the immunofluorescence procedure, and the site of the antigen–antibody reaction was revealed by fluorescein isothiocyanate (FITC) labelled sheep anti rabbit IgG (SBL Stockholm Sweden) diluted 1 20 Immunofluorescence was observed in a Leitz Orthoplan fluorescence microscope equipped with an epi illumination system An HBO 200 Hg lamp served as light source and filters were selected to give peak excitation at 490 nm (standard filter setting no 3)

For the PAP procedure the VIP antiserum used was diluted 1 5600 PAP complex (Cappel Laboratories, Downington Pa USA) was used in dilution 1 320 The sections were examined in a light microscope Sections incubated with antiserum inactivated by the addition of excess antigen (100 μg of pure porcine VIP per ml diluted antiserum) served as controls

RESULTS

Nerve fibres displaying VIP like immunoreactivity were detected throughout the upper respiratory tract The immunoreaction was blocked by adding VIP to the antiserum Generally speaking VIP nerves were most numerous in the cat, less numerous in the rabbit and rare in the guinea pig The following description of the distribution of VIP nerves applies to the cat, the distribution pattern was similar in the other species examined







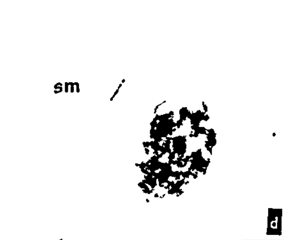


Fig 2 Cat bronchial wall Immunohistochemical demonstration of VIP (*a*) PAP stained section VIP immunoreactive nerves are present among acini of submucosal gland (×150) (*b*) VIP immunofluorescent nerves among acini of submucosal gland (×300) (*c*) VIP nerves in subepithelial connective tissue and around bundle of smooth muscle (*SM*) (×300) (*d*) Cluster of VIP immunoreactive nerve cell bodies close to smooth muscle bundle (*SM*) Note single beaded VIP nerve terminal within smooth muscle (×300)

Nasal mucosa

The maxilloturbinal area received a particularly rich supply of VIP nerves they were less numerous in the septum and upper parts of the nasal cavity Fine varicose VIP nerves surrounded the nasal glands forming dense plexa around the acini (Fig 1*a*) In addition VIP nerves were associated with fairly thick walled blood vessels (Fig 1*b*) Nerve trunks occurring in the connective tissue were occasionally seen to contain immunoreactive nerve fibres intermingled with non reactive ones

Epipharynx mesopharynx and larynx

In all areas examined scattered VIP nerves were seen around seromucous glands and small blood vessels

Tracheobronchial wall

VIP nerve fibres were most
upper parts of the

whereas they were more rare in the lower parts Fine varicose VIP nerves formed plexa around submucosal glands and small blood vessels (Fig 2*a*, *b*) VIP nerves were also observed close to and within smooth muscle bundles in the tracheobronchial wall (Fig 2*c* *d*) Single or clustered nerve cell bodies displaying VIP immunoreactivity were occasionally observed close to the smooth muscle (Fig 2*d*)

DISCUSSION

This study has revealed VIP immunoreactive nerves to be a regular constituent of the mammalian upper respiratory tract The nerves were associated with smooth muscle, sero mucous glands and blood vessels The presence of immunoreactive nerve cell bodies in ganglia within the tracheobronchial wall suggests that at least some of the VIP nerves are intrinsic to the organ, representing short pep tidergic neurons However, it cannot be ruled out that some VIP fibres in the upper respiratract may originate from distant ganglia

In the nasal mucosa and tracheobronchial wall adrenergic nerves (Dahlstrom & Fuxe, 1965, Dahlstrom et al , 1966) as well as AChE-positive nerves, interpreted as cholinergic (Ishii & Toriyama, 1972, Grote et al , 1975, Mann, 1971), have been observed in association with smooth muscle, seromucous glands and blood vessels In the nasal mucosa however, the glands seem to receive a much less prominent proportion of the adrenergic nerves (Änggård & Densert, 1974)

VIP nerves have recently been found in several types of exocrine glands, such as the salivary glands and the pancreas (Bryant et al , 1976, Larsson et al , 1976, Sundler et al , 1978, Uddman et al , 1978*b*) In the pancreas, VIP is known to stimulate secretion (Said & Mutt, 1972) Since VIP nerves occur around the seromucous glands of the nasal mucosa and the tracheobronchial wall it is conceivable that they participate in regulating the secretory activity

While vasoconstriction is thought to be mediated by adrenergic nerves the mechanism of vasodilatation is less well understood Evidence for the occurrence of non adrenergic, non cholinergic inhibitory nerves in the wall of blood vessels in various locations including the nasal mucosa, have been presented (Anggård, 1974, cf Burnstock 1975) VIP is known to be a potent vasodilator (Said & Mutt, 1970) and it is conceivable that the VIP nerves associated with blood vessels in the upper respiratory tract are involved in the regulation of local blood flow

Electrophysiological and pharmacological studies have indicated the presence in tracheal smooth muscle of inhibitory nerves which are non adrenergic and non cholinergic (Coleman 1973, Coburn & Tomita 1973) These inhibitory nerves may be identical with VIP nerves However, by immunohistochemistry another peptidergic nerve system storing substance P, has been described in the respiratory tract Hokfelt et al (1975) found such nerves in the nasal mucosa of rat and Nilsson et al (1977) observed substance P nerves within tracheobronchial smooth muscle of the guinea pig Substance P was found to cause a dose-dependent increase in the tracheobronchial smooth muscle tone (Nilsson et al 1977) In view of our finding of VIP nerves within and around the tracheobronchial smooth muscle and of the potent relaxant action of VIP on smooth muscle in other locations (Larsson et al , 1976, Uddman et al , 1978), the relaxation of adrenergically denervated tracheal muscle induced by electrical field stimulation (Coleman & Levy, 1974) may therefore reflect the action of locally released VIP rather than substance P (see also Said et al , 1974)

In conclusion the distribution pattern of VIP nerves in the respiratory tract and the above mentioned pharmacological effects of VIP suggest that such nerves are implicated in at least three physiological actions relaxation of tracheobronchial smooth muscle stimulation of secretion from seromucous glands and enhancement of regional blood flow

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ZUSAMMENFASSUNG

Nerven die VIP (vasoactive intestinal peptide) Immunoreaktivität aufweisen wurden in den Luftwanden der oberen Atmungsorgane von Meerschweinchen Karinchen und Katzen entdeckt Die VIP Nerven waren zahlreich bei Katzen weniger zahlreich bei Kaninchen und selten bei Meerschweinchen In der Nasenschleimhaut wurden feine varikose VIP Nerven gefunden die die Drusen der Nase und kleine Blutgefaße umgeben In den Wanden der Trachea und Bronchen wurden VIP Nerven in der Umgebung von seromukosen Drusen Blutgefaßen und glatter Muskulatur bemerkt Ganglien in den Wanden der Trachea und der Hauptbronchien enthielten Ansammlungen von immunoreaktiven Nervenzellkorpern die vermutlich den Ursprung der in diesem Bereich nachgewiesenen VIP Nerven darstellen

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CONCENTRATION AND DISTRIBUTION OF HEAVY METALS IN NASAL MUCOSA OF NICKEL-EXPOSED WORKERS AND OF CONTROLS, STUDIED WITH ATOMIC ABSORPTION SPECTROPHOTOMETRIC ANALYSIS AND WITH TIMM S SULPHIDE SILVER METHOD

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Abstract Biopsy specimens from nasal mucosa of 30 nickel-exposed individuals and 6 controls were analysed by atomic absorption spectrophotometry to determine the content of nickel copper cobalt zinc and iron Timm s sulphide silver staining method was used for visualizing heavy metals in cryostat sections of biopsy material from each individual The purpose of the investigation was to study the sulphide silver staining pattern in nasal mucosa of nickel-exposed individuals and controls and to establish whether variations in heavy metal concentrations especially nickel affect the histochemical pattern in the mucosa No consistent differences were found in the histochemical pattern between biopsies with high and low concentrations of nickel or any of the other metals Some difference in epithelial types between specimens from the nickel exposed group and the control group was seen but the staining pattern was quite similar for corresponding epithelial types in the two groups Two nasal carcinomas from nickel workers were virtually unstained with the sulphide silver staining method

The carcinogenic effect of nickel on experimental animals has been demonstrated by several investigators (Hueper, 1952 1958, Hueper et al , 1962, Sunderman et al 1975 Sunderman 1976)

Epidemiological studies in several nickel refineries have revealed an association between nickel exposure and development of nasal carcinomas (Morgan 1958 Mastromatteo, 1967, Doll et al 1970 Pedersen et al 1973, further references in Sunderman 1976) Torjussen et al (1976) recently demonstrated the correlation between nickel exposure and development of epithelial dysplasia, including carcinoma, of the nasal mucosa

An increased nickel content of the nasal mucosa among nickel workers has been found in an ongoing study (Torjussen et al) It is important to determine the distribution of this metal between the different tissue components of the mucosa Although several histochemical techniques have been described for demonstration of nickel and other heavy metals (Pearse 1972), most of them lack specificity (see Torjussen & Haug 1978) The sulphide silver method of Timm (1958) is probably the most sensitive method, but cannot be used to distinguish between different heavy metals Nevertheless, comparison of the sulphide silver staining pattern in specimens with normal and elevated nickel concentrations might give a clue to the site of the nickel deposition

The purpose of the present investigation was to study the concentration of nickel, copper, cobalt, zinc and iron in the nasal mucosa of nickel exposed and unexposed individuals and to investigate whether variations in these concentrations especially the nickel concentrations affect the sulphide silver pattern

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Table II *Mean values ($\bar{M}$), standard deviations (S D) and coefficients of variation (CV % metal concentrations (µg/100 g wet wt) in specimens from nasal mucous membrane of n exposed individuals, grouped according to work categories, and of controls*

	Work categories								
	Electrolysis (E)			Roasting/smelt (R/S)			Other work (O)		
Metals	$\bar{M}$	S D	CV%	$\bar{M}$	S D	CV%	$\bar{M}$	S D	CV%
Ni	270	221	82	630	673	107	124	99	80
Cu	480	290	60	627	464	74	460	291	63
Zn	2 353	1 823	77	4 657	2 480	53	2 799	3 079	110
Fe	19 552	9 915	51	25 943	9 456	36	28 737	14 967	52

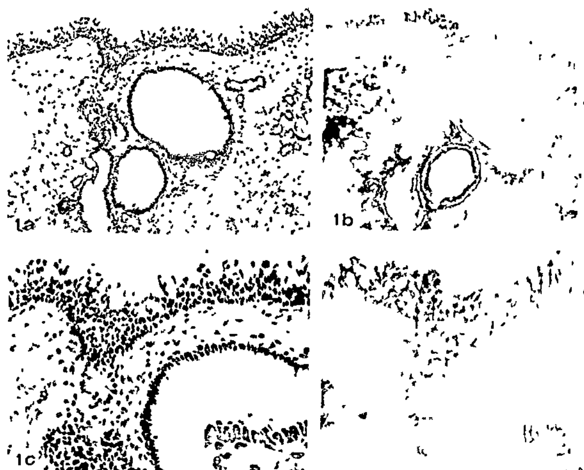


Fig 1 (a b c, d) Cryostat sections (8 µm) of specimen from nasal mucous membrane not exposed to nickel (*a c*) Stained with hematoxylin eosin and (*b d*) sulphide silver stained Stratified columnar epithelium Strong cytoplasmic staining of the basal 1-3 cell layers (*b d*) Arrows on strongly stained cells (×100 ×200) (*e f*) Glandular duct epithelium (*f*) Coarse dark are clearly seen in apical cytoplasm (*gr*) (×450)

kel-exposed duals			Controls		
	S D	CV%	M	S D	CV%
	434	123	21	16	76
	339	65	506	488	96
	2 360	77	2 117	1 289	61
	10 886	48	8 067	8 679	108

on of the specimens as shown in Table III, as made on the basis of the predominant ithelium

roma

most specimens this consisted of a normal, ose connective tissue with variable infiltration of mononuclear and scattered cells (asterisks, Fig 9a)

lands

ariable amounts of serous and mucous ands were found in most specimens Larger, perficial, glandular ducts were usually lined with the same type of epithelium as the corresponding surface (d, Fig 7a)

Sulphide Silver Staining Pattern

Surface epithelium

(1) *Pseudostratified and stratified columnar epithelium* A basal zone of 1–3 cell layers regularly showed a strong cytoplasmic staining (Figs 1b, d, 2b) In weakly stained sections the cytoplasmic stain was granular and there were often differences in stainability between individual cells (Figs 1d, 2b) In strongly stained sections the zone, except for the nuclei, appeared uniformly black (Figs 1b, d, 2b) The nuclei were largely unstained, containing only a few black granules (Fig 2b)

In general the superficial zone above the basal cell layers showed a moderate, finely granular, cytoplasmic staining (Figs 1d, 2b), while some scattered cells were distinguished by a content of larger grains (arrows Fig 1d) The secretory vacuoles of goblet cells were unstained (g, Fig 2b)

(2) *Stratified cuboidal epithelium* This epithelium usually showed the same staining as stratified columnar epithelium However, in areas of gradual changes between stratified

able III *Epithelial classification of biopsies from nasal mucosa of 30 nickel exposed and 6 on exposed individuals*

ain epithelial type the section	Number of sections (%) from persons Exposed to nickel	Not exposed to nickel
) Pseudostratified or stratified columnar epithelium	–	–
) Stratified cuboidal epithelium and some columnar epithelium	2 (7%)	1
) Stratified cuboidal epithelium	13 (43%)	4
) Stratified cuboidal epithelium and some columnar mixed or squamous epithelium	11 (37%)	1
) Mixed stratified cuboidal/stratified squamous epithelium and some cuboidal or squamous epithelium	1 (3%)	–
) Stratified squamous epithelium and some mixed or cuboidal epithelium	3 (10%)	–
otal		







Fig 2 (a b) Enlarged fields from the same sections as in Fig 1*a b* In (*b*) the superficial epithelial cells show finely granular cytoplasmic staining Goblet cells (*g*) unstained The cytoplasm of the basal cells show numerous confluent large black grains Nuclei virtually unstained but contain a few coarsely stained granules Basal cell layers together with the subepithelial connective tissue form a broad uniformly black band (*z*) Star shaped cells (*arrows*) with dense staining of the cytoplasm and of slender processes are seen in the stroma (×450)

Fig 3 Sulphide silver stained cryostat section (16 μm) of specimen no 1575 (see Table I) Stratified cuboidal epithelium is stained like stratified columnar epithelium (see Figs 1*b d* and 2*b*) The subepithelial connective tissue consists of a meshwork of stained fibre bundles oriented parallel to the basement membrane Polygonal cells with cytoplasmic staining among the fibres (*arrows*) Nuclei lightly stained (×450)

Fig 4 (a b) Cryostat sections (8 μm) of specimen no ... -eosin and ... d cuboidal ... m shows a peculiar loose spongy appearance due to numerous spaces that separate the cells from each other (*arrows* Fig 4*a*) The surface epithelium in (*b*) stains like the squamous epithelium illustrated in Fig 11*b* Strong staining of single cells and aggregates of cells in the basal epithelial layers (*arrowheads* Fig 4*b*) Superficially situated cells are occasionally strongly stained (*arrows* Fig 4*b*) (×200)

cuboidal and mixed cuboidal/squamous epithelium or stratified squamous epithelium the positive sulphide silver staining often disappeared before the change in epithelial type (Fig 5*a b*)

(3) *Mixed stratified cuboidal/stratified squamous epithelium* This intermediate type of epithelium ordinarily stained weakly like squamous epithelium When it showed staining of the basal cells this was generally weaker than in the already described types of epithelium (Fig 4*b*)

(4) *Stratified squamous epithelium* In this epithelium both the superficial and the deep layers remained virtually unstained (Figs 8*b* 9*b*, 10*b*) However some single cells in the

basal layers showed a weak, finely granular, cytoplasmic staining Occasionally, superficially situated cells were similarly stained, as found in the mixed stratified cuboidal/strati

gregates of cells in the most basal layers of the epithelium (Fig 11*b*)

Stroma

Numerous polygonal, stellate or fusiform cells showed dense staining of the cytoplasm (Figs 2*b* 3, 9*b*) and of long, slender processes, forming a meshwork between the cell bodies (Fig 3)

A finely grained intercellular staining was also seen In part this was associated with fibre bundles (Fig 3), in part more diffusely distributed Clefts, capillaries and other lumina often appeared to contain a stainable fluid (arrows, Fig 9*b*)

The subepithelial connective tissue below columnar or cuboidal epithelium often stained strongly, forming a broad dark band including also the darkly stained basal layer of the epithelium (Fig 2*b*) The basement membrane in its classical light microscopic sense, was nevertheless often discernible (arrow, Fig 2*b*) The finely grained staining associated with fibre bundles was often somewhat enhanced in the subepithelial connective tissue layer perhaps reflecting a higher density of fibres (Fig 3)

Beneath squamous epithelium the subepithelial connective tissue stained more like the remaining stroma

Mononuclear cells showed little staining (Fig 9*a*, *b*)

Glands

Glandular acini were generally unstained (as lensks Fig 7*a* *b*) Stratified epithelium in larger ducts usually stained like the surface epithelium (*d* in Fig 7*b*) In simple columnar duct epithelium coarse dark grains were found in the apical cytoplasm (Fig 1*f*)

Carcinomas

The tumour tissue showed epithelium that was virtually devoid of sulphide silver staining infiltrating in a strongly stained connective tissue stroma (Fig 12*a*, *b*)

Comparisons of the Sulphide Silver Staining between Epidemiologically and Chemically Defined Groups of Specimens

Total staining The ratings of total staining in one set of sections correlated well both for each observer and between observers ($r=0\ 7$ and 0 9, respectively, $P<0\ 01$) Moreover, similar comparisons between *adjacent* (8 μm) sections from each specimen, stained in different batches, showed good correlation ($r=0\ 7$) However, there was no correlation between the ratings of one set of 8 μm and one set of 16 μm thick sections ($r=0\ 27$) These two sets, besides being of different section thickness, were from levels spaced about 250 μm apart It is possible that the lack of correlation here reflects the heterogeneity in the blocks A comparison between specimen groups made on the basis of 'total staining" and chemical or epidemiological data, therefore seemed meaningless

Distribution pattern The staining densities of the superficial and basal layers of the different types of epithelium, of subepithelial and deeper levels of the connective tissue, of the glands and of infiltrates of mononuclear leukocytes, were rated as for 'total staining' in two 8 μm sections from each specimen We were not able to find any clearcut association between chemical and epidemiological data and the distribution and densities of the sulphide silver staining A comparison between 6 specimens with the highest and 9 with the lowest nickel concentrations showed no difference in sulphide silver pattern, nor did similar gr based on the concentrations of the ot tals on the sum of the nickel copp concentrations, or on the iron c

The specimens of the controls did not differ in distribution pattern or total staining from specimens of the nickel-exposed group, when comparing corresponding epithelial types The conclusion to be drawn here is that we failed to distinguish nickel from the other stainable metals with the present applied histochemical method

DISCUSSION

Tissue concentrations of nickel, copper, cobalt, zinc and iron in nasal mucosa

The concentrations of these metals in the nasal mucosa have not been previously investigated As regards nickel, the present data should be regarded as preliminary to a report on nickel in the nasal mucosa, plasma, and urine of nickel workers (Torjussen et al , in preparation) At this point it should be mentioned that the air in both the electrolytic and the roasting/smelting departments contains large amounts of nickel, and lesser amounts of copper In the roasting/smelting department, nickel occurs as water-insoluble compounds (nickel sulphide and oxide), while compounds with high solubility predominate in the electrolytic department (nickel chloride and sulphate) This difference in type of nickel exposure probably contributes to the significant differences in mean nickel concentration in nasal mucosa between the roasting/smelting department and the electrolytic department

The high coefficient of variation for nickel in the nasal mucosa probably reflects the variable degree of exposure to this metal in the material However, the coefficients of variations for copper, iron and zinc are also fairly high Moreover, the biopsies differ considerably with respect to the volume fractions of the different tissue types, where the sulphide silver pattern has demonstrated that the metals have an uneven allocation Thus, sampling variations may contribute significantly to the large variability of the metal concentrations

Structure of nasal mucosa

Oppikofer (1906) studied the mucosa of the in-ferior and middle turbinate in a general au-topsy material and found the same variation in epithelial types that we have described, al-though using other designations The amoun of glands and the degree of mononuclear leu-kocyte infiltration in the connective tissu varies both between and within the specimens consistent with the descriptions of Oppikofe (1906)

The present biopsies were taken from th location which deviated most from 'typical' respiratory epithelium in Oppikofer's material

Fig 5 (a b) Cryostat sections (8 μm) of specimen no 1555 (Table I) (*a*) Stained with hematoxylin-eosin an (*b*) with sulphide silver method Fold of mucosa lined with thick stratified cuboidal epithelium (*x*) Transition to stratified squamous epithelium near the top of the f l (*y*) The basal epithelial cell layers in the bottom of th fold show strong sulphide silver staining which disap-pears somewhat before the epithelium changes its ap-pearance into squamous epithelium (area between arrows (×150)

Fig 6 (a b) Cryostat sections (16 μm) of specimen no 1573 (Table I) (*a*) Stained with hematoxylin-eosin and (*b*) with sulphide silver method Obliquely sectioned Thickened stratified cuboidal epithelium penetrated by connective tissue papillae (*p*) (×150)

Fig 7 (a b) Cryostat sections (8 μm) of specimen no 1539 (Table I) (*a*) Stained with hematoxylin-eosin an

Fig 8 (a b) Cryostat sections (8 μm) of specimen no 1552 (Table I) (*a*) Stained with hematoxylin-eosin and (*b*) with sulphide silver method Counterstained with thio-nin Stratified squamous epithelium *Surface* epithelial cells are not sulphide silver stained (only counterstained) neither is the immediately subjacent subepithelial connec-tive tissue (×150)

Fig 9 (a b) Cryostat sections (8 μm) of specimen no

(*b*) is due solely to the counterstaining effect sulphide silver staining at the surface The epithelial cells are demarcated by distinct light cell borders in both sec-tions Stellate cells (*arrowheads*) with dense cytoplasm and light nuclei are seen in the stroma Capillaries and other lumina often appear to contain a stainable fluid (*arrows*) Lymphoid infiltrates (*asterisks*) in the stroma show virtually no sulphide silver staining (×150)


a
x


5b




p
6b


d
d
8b
8b



Fig 10 (a b) Cryostat sections (8 μm) of specimen no 1536 (Table I) (*a*) Stained with hematoxylin-eosin and (*b*) with sulphide silver method Counterstained with thionin Stratified squamous epithelium of unequal thickness penetrated by connective tissue papillae (*p*) The superficial and deep cell layers of the epithelium [illegible] unstained (*b*) [illegible] epithelial [illegible] are seen [illegible] by stroma in the [illegible] stained section [illegible]

This is also an area where dust and particles most easily deposit (Hadfield, 1970)

Timm's sulphide silver method

The principles and histochemical validity of this method have been described and extensively discussed (Voigt, 1951, 1959, Timm, 1958, Haug, 1973, and others) However, some important points should be recalled here

The method involves treatment of the tissue with sulphide to precipitate heavy metals As the relative amounts of metal sulphides are usually too small to be visualized, subsequent "physical development" is performed with a solution of silver nitrate and a reducing agent During this process the metal sulphides catalyse the reduction of silver nitrate to elementary silver, which precipitates in the vicinity of the sulphides It is assumed that the amounts of precipitate roughly reflect the relative amounts of metals present The method is fairly sensitive, although the lowest concentration which can be detected has not been stated (p [illegible] Haug, 1973) Furthermore, the method [illegible] a very precise spatial localization and [illegible]en applied even in electron microscopi[illegible] investigations (for references, see Torjussen et al , 1978) Unfortunately, there are no established procedures for distinguishing between different heavy metals with Timm's method, although several solutions to this problem have been suggested (Timm, 1962)

Argyrophilic substances are said to cause precipitation of silver in some tissues, without pretreatment with sulphide (Voigt, 1951, 1959) Untreated cryostat sections of nasal mucosa developed like sulphide-treated sections did not stain, however, (Torjussen et al , 1978) Sulphide treatment might create non metallic reducing groups in the tissue, but this has never been shown to give false-positive reactions with the sulphide silver method In nervous tissue this problem has been further investigated with dithizone and other chelating agents Perfusion of the brain with dithizone prior to sulphide treatment prevents all sulphide silver staining, thus attesting to the metallic nature of stainable substance in that [illegible] tion (Haug, 1973, p 52) It is probable [illegible] some metal fractions, such as iron in cy[illegible] chromes and hemoglobin, are unavailable [illegible] the sulphide However, information is [illegible] on this point, and *a priori* we must cons[illegible] the precipitate of the silver as an indicator [illegible] the presence of sulphide precipitable metals [illegible] general

Even the highest nickel concentrati[illegible] found in the exposed workers are low compared with the normal concentrations of [illegible] and zinc in many tissues, and are comparabl[illegible] to commonly found concentrations of [illegible] (Table II) We must therefore presume tha[illegible] metals other than nickel contribute most of th[illegible] sulphide silver staining in the present material Whether nickel also contributes to the visib[illegible] stain or is located in unstained areas in amounts too small to be visualized, is impossible to decide With the present concentrations of nickel, deposits will have to be concentrated and localized to structures which do not normally stain, if they are to be detected with the sulphide silver method Due to lack of more suitable methods, we nevertheless chose this procedure for the present study (Torjussen et al , 1978)

Sulphide silver staining pattern in nasal mucosa

Obviously, the heterogeneity of the nasal mucosa entails a considerable sampling problem and complicates the search for systematic variations in sulphide silver staining between

[illegible] specimen no [illegible] eosin and [illegible] with th[illegible] [illegible] helial cells [illegible] ver staining [illegible] l connective tissue shows strong staining (×150)

[illegible] m) of specimen no [illegible] n-eosin and [illegible] ned with thio- [illegible] omatous cells [illegible] nin Infiltrating nasal carcinoma [illegible] (*cc*) are virtually devoid of sulphide silver staining infiltrating in a strongly stained connective tissue stroma (*sa*) (×150)


a


11 b


sa
12a


cc
cc
cc

chemically and epidemiologically defined groups of specimens, as there are no practical procedures for quantitating and comparing staining reactions in different types of epithelium The conclusions which may be drawn at this point, are therefore general

The pseudostratified/stratified columnar epithelium generally showed intense staining of the basal cells and moderate to weak staining at more superficial levels Although the staining of stratified cuboidal epithelium usually conformed to that of the columnar epithelium, there were deviations from this pattern, where the epithelium was weakly stained throughout its thickness As such variations occurred even within one and the same section, and over very small distances along the epithelium, while the staining of the adjacent structures was uniform, they are unlikely to be artefacts of staining All these deviations in staining pattern were from specimens of the nickel exposed group

The stratified squamous epithelium was [illegible]ely unstained It is not known whether this [illegible] of Timm staining is a general characteristic of this epithelial type, or specifically reflects the chronic irritation of the nasal mucosa In the present study the squamous epithelium was found only in the nickel-exposed group

The cancerous epithelium from two nasal tumours, at least one of them diagnosed as squamous cell carcinoma, was also virtually devoid of Timm staining This seems the more interesting as speculations have been advanced that a lack of zinc may characterize certain types of cancer cells (Schrodt et al, 1964, Gyorkey et al, 1973) Voigt (1959) described a typical hormone-producing insulinoma, which did not stain with the sulphide silver staining method, even though normal Langerhans' islands contain easily stained zinc (Voigt, 1959)

As the controls did not differ significantly from the nickel-exposed group in the staining of corresponding epithelia, we conclude that the staining pattern of the columnar epithelium and its underlying stroma is a normal characteristic of the nasal mucosa This normal staining pattern is not drastically disturbed in th[illegible] stratified cuboidal epithelium However, th[illegible] formation of more squamous like stratifie[illegible] epithelium (mixed stratified cuboidal/stratifie[illegible] squamous and clear stratified squamous epithelium), appears to entail loss of Timm stainability

Despite its failure to visualize the abnorma[illegible] nickel deposits, the sulphide silver staining pattern should form a useful basis for furthe[illegible] investigation of the distribution of heavy metals in nasal mucosa

ZUSAMMENFASSUNG

Biopsien der Nasenschleimhaut von 30 nickelexponierter Individuen und 6 Kontrollen wurden mit Atomabsorptionsspektrophotometrie auf ihren Gehalt an Nickel Kupfer, Kobalt Zink und Eisen analysiert Timms Silbersulfidfarbenmethode wurde benutzt um die Schwermetalle in Kryostatschnitten sichtbar zu machen Es wa[illegible] das Ziel der Untersuchung das Silbersulfidfärbemuster in der Nasenschleimhaut von nickelexponierten und Kontrollen zu studieren und zu sehen ob Unterschiede im *Schwermetallgehalt – insbesondere* von Nickel – das histochemische Farbemuster beeinflußten Es konnten keine konstanten Unterschiede im histochemischen [illegible] mit hohem oder niedrigem Ge[illegible]

[illegible]

funden Aber die histochemischen Farbemus[illegible] sehr ähnlich fur die entsprechenden Epitheltypen der beiden Gruppen Zwei Nasenkarzinome von Nickelar[illegible] beitern waren so gut wie ungefärbt nach Anwendung der Silbersulfidfarbemethode

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THE CENTRAL RHYTHM OF THE NASAL CYCLE

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Abstract The resistance to air flow of each nasal passage was recorded in 2 subjects over a period of 7 days The cyclical changes in nasal resistance observed were very regular, with a consistent pattern apparent over the period of study The regular changes in nasal resistance recorded under laboratory conditions may be directly related to changes in activity of an autonomic centre in the brain

The spontaneous changes in the resistance to air flow of each nasal passage associated with the nasal cycle are believed to be regulated from a nasal centre in the hypothalamus or medulla A hypothalamic centre regulating the ' cycle was first proposed by Stoksted in and in 1959 Malcomson demonstrated stimulation of the cat hypothala caused a nasal vasoconstriction A nasal associated with the respiratory centre in the medulla has also been suggested as changes in nasal resistance are often associated with changes in respiration (Dallimore & Eccles, 1977) The nasal resistance is influenced by many factors in the environment but under controlled laboratory conditions one might expect the changes in nasal resistance to be directly related to an endogenous rhythm in the nasal centre The present study is concerned with demonstrating the marked regularity of the nasal cycle under laboratory conditions and provides further evidence for a nasal centre as the regulator of the spontaneous changes in nasal resistance

METHODS

Experiments were performed on 2 male subjects R E (the author, age 28) was of British origin and had been exposed to the Indian climate in Delhi for 2 weeks prior to the experiments, and A K (age 25) was an Indian resident of Delhi Both subjects were healthy and were not prone to any allergic disorders or chronic nasal or sinus infections There were no external signs of a deviated nasal septum and no previous history of trauma to the nose or paranasal sinuses in either subject

The resistance to air flow of each nasal passage was determined by measuring the pressure and flow characteristics associated with normal breathing and plotting the pressure against flow by display on a storage oscilloscope The slope of the plot on the oscilloscope screen between flows of ±10 l/min was determined by fitting a straight line to the graph and the nasal resistance was expressed in units of cm H_2O/l/sec (R) Only the flow rates between ±10 l/min were used for the calculations as at higher flow rates the air flow in the nasal passages is not laminar and the resistance is not a direct linear function of pressure and flow In the present experiment the formula $R=\Delta P/V$ described the relationship between pressure, flow and resistance

To measure pressure and flow a soft plastic cannula was inserted approximately 0 5 cm into each nostril The cannulae were 22 cm in length with an internal diameter of 0 9 cm and a wall thickness of 2 5 mm

A cannula from one nostril was connected to a pneumotachograph cone to measure air flow (V), with a differential pressure transducer (Hewlett Packard model 270) to determine the pressure change across the cone The signal from the transducer was fed into an amplifier (Hewlett Packard 88058) and then

played on the horizontal axis of a storage illoscope (Tektronix type 5103N)

The cannula from the other nostril was con ted to one arm of a differential pressure nsducer (Statham PM 283 TC) and this asured the nostril pressure which under the ditions of the experiment was equal to the tenor nares pressure as no air flow oc red in this cannula The other arm of the sducer was connected to a large bore nge needle inserted into the other cannula d to record air flow, and the pressure was sured close to the nostril With this ar gement the pressure change from the pos or nares to the nostril could be measured) The signal from the transducer was fed an amplifier (Statham bridge amplifier I SC 100) and then to the vertical axis of the age oscilloscope

Vith the cannulae inserted into the nostrils pressure and flow relationships of one na passage during normal respiration were ted on the oscilloscope, and then the can e swopped around and the other nasal age monitored

he resistance of both nasal passages in subject was determined at approximate-) min intervals throughout the day (09 00– 0) The subjects remained in the labora for the duration of the experiment each and avoided any exertion The tempera range for the duration of the study was een 25–33°C and the relative humidity be n 64–98% saturated

RESULTS

changes in nasal resistance of the sub for 3 consecutive days of recording are n in Figs 1 and 2 Both subjects had reg cyclic changes in nasal resistance with a ite reciprocal relationship between the tance of left and right nostrils

e cyclic changes in nasal resistance for ct R E shown in Fig 1 are remarkably ar for the 3 days illustrated At the start of rding in the morning the left nasal passage has a high resistance and the right nasal pas sage a low resistance with most of the air flow occurring through the right nostril The changeover from right to left nostril dominance of air flow occurs at about 11 30 hr with further crossover points at 14 00 and 16 00 hr The crossover points for 3 days of recording are joined with a straight line through the graphs to demonstrate the regularity of the cycle

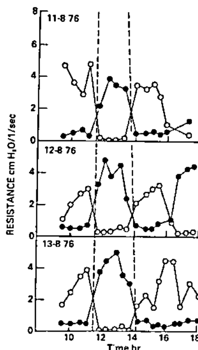


Fig 1 Changes in nasal resistance in subject R E for 3 consecutive days O Left nasal passage ● right nasal passage

The Indian subject A K showed similar regular changes in nasal resistance yet the crossover points between left and right domi nance showed some phase shift each day as demonstrated by the straight lines drawn through the crossover points on the three con secutive graphs (Fig 2) In both subjects the crossover points could not be correlated with the taking of food or drink or any change in the laboratory environment

The changes in reistance were similar for both subjects with the maximum resistance a nasal passage varying between 4–6 cm l/sec and the minimum resistance

0 02–1 0 cm H_2O/l/sec, depending on the phase of the cycle

The period of the cyclic changes in resistance in the two subjects varied between 1–2½ hr between crossover points depending on which nostril was dominant Subject R E had a very regular cycle with the time periods of left or right dominance of approximately equal duration giving a crossover approximately every 2 hr

The times of the crossover points of the graphs of the nasal cycle are tabulated in Fig 3 for the seven days of recording Subject R E has a very regular pattern of crossover points with changes in resistance at 11.00 and 14 00 hr which are consistent for the 7 days of recording and unaffected by the weekend break on the 14th and 15th of the month The changes in resistance which occur after 14 00 hr do not have the same regular pattern

The crossover times for subject A K as shown in Fig 3 appear to have a random distribution on first inspection yet a regular phase t in the period of the cycle is apparent on examination, similar to that observed in)

e total nasal resistance calculated for oth nasal passages in parallel varied according to the phase of the cyclic changes in resistance The changes in total nasal resistance for subject R E are shown in Fig 4, where it is apparent that there is some regularity in the changes in resistance from day to day, with a range in nasal resistance from 0 06–0 64 cm H_2O/l/sec Subject A K showed similar changes in total nasal resistance with a range from 0 21–1 14 cm H_2O/l/sec during the three days of recording

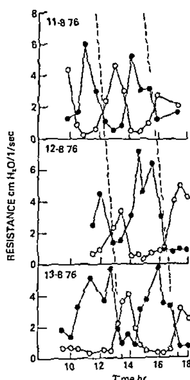


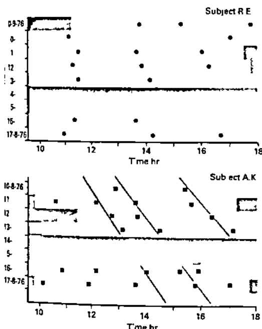
Fig 2 Changes in nasal resistance in subject A K for 3 consecutive days O Left nasal passage ● right nasal passage

DISCUSSION

The results demonstrate that there are regular reciprocal changes in nasal resistance in both the subjects studied Similar results have been previously reported, but other workers have not recorded the changes in nasal resistance in the same subjects on consecutive days

In subject R E the changes in resistance of each nasal passage occur at approximately the same time each day (11 00 and 14 00 hr) with no apparent phase shift The changes in nasal resistance which occur after 14 00 hr are not as consistent from day to day as the crossover times observed at around 11 00 and 14 00 hr If the nasal cycle was in some way connected with the time of rising from sleep and this time was consistent each day then it is possible that the cycle could be set by this time and gradually drift from the set point towards evening The daily changes in nasal resistance which occurred at around 11 00 and 14 00 hr were not related to any intake of food or drink as meal and snack times were alternated in both subjects

The nasal cycle of subject A K shows a significant change in the time of crossover each day yet this is probably a regular phase shift, although it would be necessary to record over a longer period to confirm this result



g 3 A plot of the crossover times of changes in nasal sistance The shaded areas represent the periods during ch recordings of nasal resistance were not made

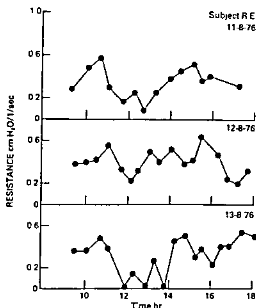


Fig 4 Changes in total nasal resistance in subject R E for 3 consecutive days

The phase shift each day may be explained the relationship between the time period of e cycle and whether or not it is divisible to a 24 hr period If the period of the cycle exactly divisible into 24 hr then the cross er times will occur at exactly the same time ch day, and if the period is not divisible into hr then there will be some phase shift each y The regularity of the nasal cycle from y to day indicates that the cyclic changes nasal resistance occur throughout the 24 hr riod and that changes in nasal resistance ay continue during sleep

The total resistance of the nasal passages air flow has been previously stated to re- ain relatively constant during the reciprocal anges in the resistance of each nasal pas ge (Stoksted 1953) The results of the pres t study show that the total nasal resistance ay vary according to the phase of the cycle subject R E the total nasal resistance nged from 0 06–0 64 cm H_2O/l/sec and in bject A K from 0 21–1 14 cm H_2O/l/sec e variation in total nasal resistan[illegible] ably associated with an undetected asymmetry of the nasal passages

The functional significance of the cyclic changes in resistance is obscure, the changes in the state of the erectile tissue have been noted to coincide with changes in the secre tory activity of the nasal mucosa (Lillie, 1923), and it is possible that there is periodic activity of the nasal mucosa in conditioning the inspired air, with one nasal passage under- going a rest period whilst the air flow is di rected through the other passage

The vasomotor and secretory activity of the nasal mucosa is regulated by the autonomic nervous system (Änggård & Edwall 1974, Eccles & Wilson 1974, Malm 1973), and the changes in activity of autonomic outflow which occur during the nasal cycle must be regulated centrally The hypothalamus has been proposed as an area of the brain most likely to be involved in regulation of the nasal cycle (Stoksted 1953) and experiments on the cat have demonstrated that electrical tion of the hypothalamus causes a nasal resistance (Malcomson 1959)

the cyclic changes in nasal resistance are very regular when recorded under laboratory conditions, probably because the control of nasal resistance is then dominated by the endogenous nasal centre Normally the activity of the nasal centre might be expected to be influenced by many factors such as, exercise and arterial pCO_2 (Dallimore & Eccles, 1977), emotion (Wolf, 1954) and skin temperature changes (Drettner, 1961) When changes in these factors are limited then the central rhythm of the nasal cycle is apparent and this is very regular from day to day

Persons trained in *pranayama* (breathing exercises in Yoga) are able to alter their nasal air flow at will from one nasal passage to the other and presumably they have developed conscious control of the autonomic nasal centre Ancient documents relating to the practice of Yoga describe changes in nasal air flow as related to changes in the state of meditation and mood (Sing & Chhina, 1974) and if e nasal centre is indeed located in the hypo mus as proposed by Stoksted then there y be a physiological basis to these ancient liefs, with changes in mood affecting the activity of a hypothalamic nasal centre

SUMMARY

1 Cyclic changes in nasal resistance have been observed in 2 subjects over a period of 7 days
2 The period of the nasal cycle was regular, with a consistent pattern apparent for the changes in resistance throughout the 7-day study
3 The total nasal resistance of each subject varied according to the phase of the nasal cycle
4 The regular changes in nasal resistance recorded under laboratory conditions may be directly related to changes in activity of an autonomic nasal centre in the brain

ZUSAMMENFASSUNG

Bei zwei Versuchspersonen wurde während einer Zei von sieben Tagen der Luftstromwiderstand in den beidei Nasengängen registriert Die beobachteten zyklische Veränderungen des Nasenwiderstandes wiesen ein seh regelmäßiges und während der Beobachtungszeit gleich bleibendes Muster auf Die unter Laborbedingungen re gistrierten regelmäßigen Veränderungen des Nasenwider standes könnten in direkter Verbindung mit Veränderun gen der Aktivität eines autonomen Zentrums im Gehir stehen

ACKNOWLEDGEMENTS

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CHARACTERIZATION OF CLINICAL TONSIL STAGES BY THEIR T-CELL COUNT

Immunopathological Description of Tonsils after a Peritonsillar Abscess

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mmary The T-cell counts in five different clinical tonsil ges are compared No particularities were observed th the exception of the condition after a peritonsillar scess The differences existing between the individual ups are discussed as age-dependent A considerable in-ase in T cells is found in the abscess tonsil compared th other tonsils and the T-cell count in blood Its patho-chanism is discussed

ı important advance ın immunologic re-arch is signified by the possibility of dif-entiating between B lymphocytes and lymphocytes due to different surface mark-s It is now possible to analyse the immuno-gic stage of lymphoid cell populations and eir changes The E-rosette formation with eep erythrocytes was used for the determi tion of T cells (Brain et al , 1970, Jondal et , 1972, Lay et al , 1971)

Numerous publications are already avail le on the T-cell content of the tonsil (Yata al , 1973, Bellanti et al , 1974, Brown & eaves, 1974, Greaves et al , 1974, Greaves Brown, 1974, Tabata et al , 1974, Watanabe al , 1974, Yu et al , 1974, Hurtado et al , 74, Holowiecki et al , 1975, Ishikawa et al , 75, Morag et al , 1975, Strelkausas et al , 75, Alexopulos et al , Sugiyama et al , 1976, illson et al , 1976) Due to different cell olation methods test modifications, evalua n methods and groups of patients, values e found between 21% and 74% which are actically impossible to compare one with an her Clinical aspects concerning the stage of e tonsil, however, are not considered in the investigations The present study aims to cor-relate the T-cell changes of the tonsil with sev eral clinical conditions of the organ For this reason we have determined the T cell count in tonsillar cell suspensions and peripheral lym phocytes in a group of 86 patients

MATERIAL AND METHODS

Obtaining of material, cell isolation, and fine purification of lymphoid cells from tonsils and blood have already been described in detail (Siegel, 1978*b*) and are, for this reason, only briefly outlined here

The tonsils were cut into pieces with scis-sors, and the tissue pap shaken in Hanks' solution Tissue residues were removed by filtration through gauze, the suspension cen trifuged at 160 *g* for 10 minutes and placed in Parker's solution

Lymphoid cells were isolated from the sus-pension and blood through a gradient of Viso-trast/Ficoll having the density of 1 085 in ac-cordance with the method of Bøyum (1968) Blood was taken directly before or after ton-sillectomy by venipuncture and 50 I U /ml preservative free heparin was added

E rosette assay

In accordance with the method of Jondal et (1972) a 50 μl suspension of 3×10^6/ml dient purified tonsillar cells or blood !

Table I *Average of the T-cell number in different disease groups*

Group	Tonsil (%)	Blood (%)	*n*
1	36 1±10 6	57 0± 7 8	31
2	42 8±13 1	63 4± 9 9	7
3	48 5±10 0	61 6± 8 3	18
4	47 8±13 2	58 7± 8 1	17
5	60 0±15 8	58 0±11 9	13

C [illegible]
C
C
C
C

With the exception of Gr 5 the differences observed in the tonsil are conditioned by age The T cell count is always below that of blood In group No 5 the value is significantly above the values of the other groups and also of blood There are no differences in T cell numbers of blood between the different groups

cytes in Parker's solution was mixed with a 50 μl suspension of sheep erythrocytes having a cell count of 10^8/ml in physiological saline The sheep erythrocytes were 1–4 days old and ashed twice in physiological saline directly use After a 5 minute preincubation at the cells were sedimented at 50 *g* for minutes and incubated in a refrigerator for one hour Then the cells were resuspended by careful rotation and transferred into a counting chamber The number of rosettes was determined by counting of 100 lymphoid cells

RESULTS AND DISCUSSION

The group of 86 patients covered the age range 2 to 57 years This group was divided according to the clinical stages of the tonsils as follows

1 hyperplastic tonsils of children and young people (*n*=31) aged 2 to 17,
2 atrophic tonsils (*n*=7) of children aged 4 to 9,
3 chronically inflamed tonsils of adults (*n*=18) aged 11 to 34,
4 tonsils of adults with suspected focal infection (*n*=17) aged 10 to 40,
5 tonsils after a peritonsillar abscess in children and adults (*n*=13) aged 4 to 57 (interval tonsillectomy, for individual data see Table II)

The results of the investigations are shown in Table I in the form of averages of the individual groups It can be seen that the tonsils T-cell count is always below that of blood with the exception for abscess tonsils W as the values of all groups are practically constant in blood, there are differences between

Table II *T-cells after a peritonsillar abscess*

No	Age (years)	Sex	Abscess (%)	Opposite side (%)	Peripheral lympho-cytes (%)	Time between abscess and operation (weeks)
1	4	♂	49	35	50	6
2	10	♀	52	31	45	8
3	14	♂	36	19	59	6
4	15	♀	66	50	62	7
5	19	♀	88	64	58	6
6	19	♀	64	43	60	6
7	20	♂	44	35	41	9
8	22	♀	73	51	59	8
9	27	♂	43/44	–	3[illegible]	5
10	30	♀	86	74	72	6
11	35	♀	75	70	73	6
12	43	♀	5[illegible]	53	71	8
13	57	♀	68	64	72	6

T-cell number in the abscess to [illegible] the contralateral tonsil and in peripheral lymphocytes Increased values are found on the side affected by the absc[illegible] [illegible]mpared with peripheral lymphocytes The contralateral side shows only partly pathological changes and the valu[illegible] [illegible] [illegible]ot exceed those of blood The tonsillectomy was done normally at an interval of 6 weeks after the abscess

e individual clinical groups However, the fferences are not significant in groups of ap oximately the same age and the existing difrences of groups 1 and 2 compared with oups 3 and 4 are, above all, conditioned by e and not by the clinical stages Elsewhere were able to demonstrate a relationship tween the tonsils' T cell count and age (Sie- l 1978*a*) The group of tonsils after a perinsillar abscess shows a different behaviour ie group average here exceeds that of blood ie values of this group are listed individually Table II A distinction is made between the scess tonsil and the non affected tonsil acrding to case history details It can be seen at the contralateral tonsil frequently shows increased T cell count, but it does not raise e values of the abscess side The normal beviour of Pat No 3 values in pat no 3 is esumably based on insufficient case history tails and the atypical behaviour of Nos 12 d 13 can possibly explained as advanced nsil involution in the early stages of the abess

An interpretation of the T cell accumulation the abscess tonsil is possible in connection th other analytical data Whereas exclusion T cells is normally observed in the tonsil mpared with the values of blood, the tonsil ows a contrary behaviour after an abscess In previous investigations we were able to monstrate a considerably reduced uridine corporation in this stage, which was intereted as a decrease of cell proliferation (Sie l 1978*c*)

A decrease in the RNA and DNA conntrations along with an increase in the proin/DNA ratio was also shown (Siegel & ilke 1975) Since mainly B cells proliferate the tonsil, according to the investigations of olowiecki et al (1975) a relative concentra n of T cells takes place in the abscess tonsil we observed a reduction of the proliferative tivity in the organ An atypical composition lymphoid cells occurs Owing to the reducn in proliferation activity the tonsil cannot lfil its amplifier function in the sequence from antigen contact to immune response This should be considered in the indication for a tonsillectomy after a peritonsillar abscess, even in chilcren In the groups listed in Table I, no further pathological particularities were apparent, except in the stage after a peritonsillar abscess From this we conclude that the scattering of T cells is larger in the tonsil due to processes of natural involution than by pathological processes The individual variation, however, is considerable

ZUSAMMENFASSUNG

Die T Zell Zahl bei 5 verschiedenen klinischen Tonsillen stadien wird verglichen Mit Ausnahme des Zustandes nach Peritonsillarabszeß wurden keine Besonderheiten beobachtet und die zwischen einzelnen Gruppen be stehenden Differenzen als altersbedingt diskutiert Bei der Abszeßtonsille tritt eine starke Zunahme der T Zellen im Vergleich mit den ubrigen Tonsillen und der T Zell Zahl des Blutes auf Sein Pathomechanismus wird diskutiert

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THE DNA CONTENT AND NUCLEAR SIZE IN NORMAL, DYSPLAS[illegible] AND CARCINOMATOUS LARYNGEAL EPITHELIUM

A Spectrophotometric Study

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[Ab]stract The aim of the present study was to obtain [a] more objective evaluation of nuclear hyperchromasia [an]d polymorphism in laryngeal epithelium The method [is] based on a photometric assay of nuclear size and DNA [co]ntent in Feulgen stained slides The DNA content of [hy]perchromatic nuclei should exceed the mean for nuclei [in] normal epithelium by more than twice the standard [de]viation In comparison with lymphocytes (2N) the [DN]A content in hyperchromatic nuclei corresponds to 3 N The mean DNA contents of normal dysplastic [an]d carcinomatous laryngeal nuclei were 69 71 and 118 [A]U The mean nuclear area for normal dysplastic and [ca]rcinomatous epithelial cells were 48 41 and 73 μm^2 [T]he higher the degree of atypia displayed by the cells [th]e greater was the variability of the DNA content and [th]e nuclear size The investigation shows that the method [m]ay provide a more objective basis for evaluating hyper[ch]romasia and polymorphism

[T]he histopathological grading of atypia in la[r]yngeal epithelium is based on morphological [cri]teria that are occasionally subtle and thus [li]able to individual differences in evaluation [A] more accurate assessment of atypias is re[q]uired for both diagnostic and therapeutic pur[p]oses Nuclear hyperchromasia and polymor[p]hism are two important parameters in the [g]rading of atypia in squamous epithelium

The importance of nuclear polymorphism in [t]he malignancy grading and prognosis of [g]lottic carcinoma has been well documented [b]y Jakobsson (1973)

Nuclear hyperchromasia—that is, an en[h]anced stainability of the nuclei—is due to the [p]resence of increased amounts of acidophilic [s]ubstances, including DNA, RNA, histones and other proteins

Normal nuclei may also be hyperchromatic when passing the S and G2 cell cycle phases However, the extent to which the individual increase in DNA, RNA, histones and other proteins is responsible for the hyperchromasia observed in cells stained by the haematoxylin and eosin method has not hitherto been established Eneroth et al (1974) also showed significantly increased DNA values in Feulgen-stained cells from an acinic cell tumour without malignant histological features

Cells with abnormal chromosome numbers are referred to as aneuploid (or heteroploid) It would perhaps be more appropriate to refer to the chromosome constitution as hyperdiploid, hypodiploid etc thus indicating more precisely the deviation from the diploid constitution The chromosomes may, however, be present in multiples of the haploid number, thus giving rise to triploidy (3*N*), tetraploidy (4*N*) or higher degrees of ploidy (Koller, 1972)

The nucleoproteins consist of proteins and nucleic acids, to which they owe their staining properties The best available histological staining method for DNA, is based on the Feulgen reaction, which is specific and does not produce staining of RNA (Feulgen & R[o]senbeck, 1924, Disbrey & Rack, 1970)

The histopathological concept of polymorphism is based on va[riations in] size and shape of the nuclei and [distribu]tion of chromatin within the

ject of the study reported here was to find whether a photometric examination with determination of the DNA content and size of the nuclei in atypical laryngeal epithelia might afford a more precise foundation for grading of hyperchromasia and nuclear polymorphism

MATERIAL AND METHODS

Microscopic preparations

Nine laryngeal biopsy specimens, one from each of 9 patients, were selected and classified in the following way

Cases 1, 2 and 3 Normal squamous epithelium, with neither hyperplasia nor atypia

Cases 4, 5 and 6 Squamous dysplasia—mild, moderate and severe, respectively

Cases 7, 8 and 9 Squamous cell carcinoma moderately well differentiated

The specimens were fixed in 10% neutral buffered formalin and embedded in Paraplast Plus (Sherwood Industries)

n addition, lymphocytes were studied in rs from blood and from imprints using the e fixative

Histological sections

Sections 8 and 14 μm thick were cut with a motorized microtome with a vertical knife (Leitz microtome Model 1212)

Feulgen reaction

All specimens were stained simultaneously according to the Feulgen method (Feulgen & Rossenbeck, 1924, Disbrey & Rack, 1970) with hydrolysis in 1 N hydrochloric acid at 60°C for 12 minutes and stained with para rosaniline (C I 42500) The sections were mounted with Diatex (refractive index 1 485) and stored in the dark at room temperature

Photometry

The photometric measurements were carried out at the wavelength of 547 nm using a Leitz microspectrophotometer (Leitz Sincel 8 programme), MPV II connected to a compute PDP 8E (Digital Equipment) With this equip ment a rapid scanning can be performed with measuring spot of 0 25 μm² in area (Nord strom et al , 1975)

The accuracy was increased by making 1 registrations of transmission at each measu ing spot The absorbance was obtained b applying Beer Lambert s law using the loga rithm of the mean The area of the cell nucle was estimated from the number of measuri spots registrated within the nucleus

Conditions and errors in photometric measurements

The theoretical and practical bases of phot metry have been discussed in detail by sever authors (Caspersson et al 1957, Glick et a 1951, Leuchtenberger 1958)

1 In photometric measurements it is esse tial that the absorbance be proportional to t concentration and the thickness of the absor ing substance so that Beer Lambert s law valid

2 For the quantitation of DNA by the stai ing reaction in question the reaction must a stoichiometric one (Glick et al 1951)

3 The (distributional) error incurred by heterogeneous distribution of the absorbi substance has been calculated and critica examined by a number of workers (Glick al , 1951, Garcia 1962*a*, Leuchtenberg 1958) For normal nuclei it has been estimat at about 1% and for carcinomatous nuc at about 5–10% (Sandritter et al , 1960) T distributional error increases with the absor ance (Garcia, 1962*b*) but this error can diminished by using a wavelength that gi a lower absorbtivity (Leuchtenberger 1958

4 Light scatter, another source of err was reduced by inserting a special diaphra in the optical system

5 A high level of reproducibility of t photometric measurement is essential (fi measurements of the same nucleus gave a efficient of variation of 1 02%)

6 The distribution of the absorbing p

ble I *Mean DNA absorbance (content) and area of cell nuclei and the standard deviation d coefficient of variation for 8 and 14 µm sections*

	8 µm			14 µm	
Case	Mean±S D		Coefficient of variation	Mean±S D	
Absorbance (A U)					
Normal					
1	70 6± 7.5			70 9± 8 9	
2	68 0± 8 4	69 2± 8 3	12 0%	69 1± 7 7	70 3± 8 6
3	69 0± 8 9			70 8± 9 3	
Dysplasia					
4	71 6±23 8			72 4±26 1	
5	70 2±25 4	71 5±23 8	33 3%	71 1±26 4	71 8±25 4
6	72 6±22 2			71 9±23 7	
Carcinoma					
7	97 8±41 8			99 2±44 1	
8	138 1±74 1	117 5±57 5	48 9%	159 4±78 9	129 1±61 4
9	116 6±44 4			128 7±61 0	
Area (µm²)					
Normal					
1	55 2±11 8			53 8±10 7	
2	44 4± 9 6	47 7±11 3	23 7%	45 0± 9 9	48 0±10 5
3	43 5± 8 4			45 3± 8 9	
Dysplasia					
4	46 9±16 1			45 7±17 2	
5	40 3±14 9	41 4±15 4	37 2%	41 2±14 0	41 6±16 0
6	37 0±13 7			38 0±14 2	
Carcinoma					
7	70 6±22 9			76 4±24 0	
8	82 6±43 6	73 3±34 8	47 5%	92 7±42 1	87 6±35 1
9	66 7±28 3			78 6+30 3	

ticles depends to some extent on the type of fixative used Some produce coarser clumping than others (Greisen, 1971) In this laboratory 10% neutral buffered formalin has been found to be the best fixative (Hellquist et al 1975)

7 The processing method must be stable By measuring lymphocytes from blocks embedded and stained at different times the reproducibility of the staining reaction could be controlled

8 A consistent thickness of the sections is another important requirement If the number of measurements is large enough the thickness of the sections can be regarded as a statistical variable and by using the mean any fluctuations in thickness will then be eliminated (Greisen 1971)

RESULTS

I *Sections thickness 8 µm*

Measurements on 75 interphase nuclei per sample (Table I)

Normal laryngeal squamous epithelium (Cases 1–3) A histogram showing the DNA content of normal epithelial nuclei and lymphocytes is presented in Fig 1 A The mean DNA content of normal epithelial nuclei was 69 2 arbitrary units (A U) The range was fairly small, the coefficient of variation being 12 0% The mean area of the nuclei was 47 7 µm², with a coefficient of variation of 23 7% Comparison of the DNA content and the area of the nuclei gave a coefficient of correlati of 0 56 (Table II)

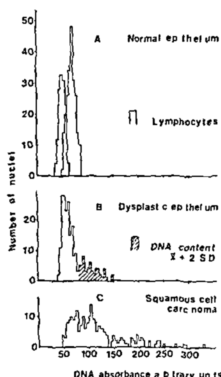


Fig 1 Frequency histograms of nuclear DNA absorbance (content) A normal epithelium B *dysplastic epithelium* C squamous cell carcinoma Section thickness 8 μm =mean absorbance value for normal epithelium (69 2 U)

Dysplastic epithelium (Cases 4–6) The DNA distribution for nuclei of dysplastic epithelium differed from that for the normal nuclei (Fig 1B) The mean DNA content 71 5 A U was not increased but there was a large variability about the mean The standard deviation was 23 8 A U giving a coefficient of variation of 33 3%

The mean area of the nuclei was 41 4 μm^2, and the coefficient of variation 37 2%

The coefficient of correlation between the DNA content and the area of the nuclei was 0 79 (Table II)

Squamous cell carcinoma (Cases 7–9) The histogram for the distribution of the tumour nuclei by DNA content shows a wide range of ploidy class (Fig 1C) Although there is a tendency towards aneuploidy there is a large class around the tetraploid value Both the mean and the variation were increased with values of 117 5 A U and 48 9% respectively

The mean nuclear area of 73 3 μm^2 impli a greater size than for either normal or dy plastic epithelium The coefficient of vari tion was 47 5% The coefficient of correlatic for DNA content versus area was as high 0 86 (Table II) this points to a close relatio ship between nuclear size and DNA content

II *Sections thickness 14 µm*

Measurements on 15 interphase nuclei p sample (Table I)

Normal dysplastic and carcinomato epithelium (Cases 1–9) The area and D content of nuclei of the normal and dysplas epithelium were about the same in the 14 µ and the 8 µm sections On the other ha the nuclear area in squamous cell carcinom was increased

III *Lymphocytes* (Fig 1 A and Table III)

(*a*) Sections of thickness 8 and 14 µm (Ca 1–9) Measurements on 15 lymphocy per sample
(*b*) Smears (i) Three imprints Measureme on 15 lymphocytes per sample (ii) Blo Measurements on 25 lymphocytes

There was no difference in the mean D content for the 8 and 14 µm sections was there a significant difference betw sections and smears

Table II *Coefficient of correlation in lin regression of nuclear DNA absorbance (c tent) and area (µm²)*

Case	Coefficient of correlation	
Normal		
1	0 57	
2	0 55	0 56
3	0 71	
Dysplasia		
4	0 86	
5	0 85	0 79
6	0 71	
Carcinoma		
7	0 85	
8	0 89	0 86
9	0 84	

le III *Mean values of the DNA absorb-e (content) of lymphocytes*

15 ∇n=25

	Histological sections		Smears	
	8 μm Δ	14 μm Δ	Imprints Δ	Blood ∇
	52 2	53 0	52 1	51 9
	5? 4	51 1	51 8	
	51 5	53 0	51 0	
	50 3	51 2		
	50 6	52 2		
	51 8	51 4		
	52 0	51 2		
	53 2	52 5		
	51 5	53 2		
	51 7	52 1	51 6	51 9

he mean DNA content for 8 μm sections, ely 51 7 A U , was taken as the reference the diploid value (2N)

DISCUSSION AND CONCLUSIONS

histological grading of atypia is based on mparison with normal epithelium, and the luation contains a large subjective element rder to achieve a higher level of objectivity he classification a photometric evaluation he DNA content and the variation in nu r size was performed using a Leitz scan photometer

photometric examination of histological ions it is necessary to identify undamaged lei that represent the relevant histopatho ical changes Since it is very difficult to npletely exclude nuclei that have been cut the microtome photometry of histological tions is not a strictly quantitative method does however have the advantage that examination can be performed on repre tative portions of the specimens

-ymphocytes are considered to have a loid DNA content in this study the value 75% of the mean for normal epithelial lei While this difference cannot at present adequately accounted for, it is consistent with the results reported by other investigators (Atkin & Richards, 1956, Atkin, 1964, Bader et al , 1960, Wagner et al , 1967) The photometric parameters of atypias were accordingly compared with those for normal mucosa The mean DNA content of normal laryngeal epi thelium, namely 69 2 A U (Cases 1–3) is referred to not as 2N but as $\bar{x}$

The fact that the size of the nuclei in normal and dysplastic epithelium was not greater in the 14 μm than in the 8 μm sections suggests that the latter displayed no effect of cutting Furthermore, the nuclei in dysplastic epithelium were essentially the same size as those in normal epithelium, a comparison can there fore be made The carcinoma cell nuclei (Cases 7–9), on the other hand, were larger in 14 μm sections, and some of them may therefore have been cut in 8 μm sections, this may have affected the absorbance values (Table I, Fig 1 C)

When the DNA content exceeds the mean by more than twice the standard deviation—corresponding to 3 3 N—the nuclei were con sidered to be hyperchromatic The absence of hyperchromatic nuclei in normal laryngeal epithelium (Fig 1 A) is consistent with the absence of DNA synthesis above the para basal layer (Fabrikant & Cherry, 1969) In dysplastic and carcinomatous laryngeal epi thelia on the other hand hyperchromatic nuclei are numerous (Fig 1 B, C) which is also found by Greisen (1975) and Giarelli et al (1977)

So long as the mean DNA content does not fall significantly below its normal value an increase in the coefficient of variation implies the presence of nuclei with an elevated DNA content Thus, another criteria of the hyper chromasia in a cell population may be the co-efficient of variation of the DNA content The coefficient of variation for the nuclear area was markedly higher for nuclei of dysplastic epithelium than for nuclei of normal epithe lium It would thus seem that the variability of both nuclear size and DNA content in creases with the degree of atypia

Comparison of each cell nucleus

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Conferences and Meetıngs

1978, Nov 23–26 Course on Septoplasty mıcrosurgıcal aspects and endonasal approach of the pterıgomaxıllary fossa and annexed cavıtıes" wıll be held ın Barcelona Addr Secretary of the Cours, Muntaner, 366-3°, Barcelona 6 Spaın

1979 Jan 15–19 The Fourteenth Annual Otologıc Surgery Course wıll be held at The Ear Research Instıtut ın Los Angeles Addr F H Lınthıcum, Jr M D , Ear Research Instıtute, 256 South Lake Street, Los Angeles Calıfornıa 90057, USA

1979, March 4–9 Department of Otolaryngology, Unıversıty of Toronto announces Wınter Meetıng 1979—Mont Trembland Lodge Addr Dr Wıllıam Crysdale Suıte 6126, 555 Unıversıty Avenue, Toronto Canada M5G 1X8

1979 Aprıl 29–May 4 Thırd Internatıonal Symposıum on Plastıc and Recon structıve Surgery of the Head and Neck to be held ın New Orleans Addr Jack R Anderson, M D , 1111 Tulane Avenue, (Ste 322) New Orleans Louısıana 70112, USA

1979 Aprıl 1–6 The Asıa Oceanıa Assocıatıon of Oto-Rhıno-Laryngologıcal Socıetıes Fourth Asıa Oceanıa Congress to be held ın Sydney, Australıa Addr The Congress Secretarıat GPO Box 2609, Sydney, NSW, Australıa 2001

1979 Aprıl 22–28 The Department of Otolaryngology, Unıversıty of Nıjmegen the Netherlands, announces the 14th Post Graduate Course ın Ear Surgery Addr Dr W F B Brınkman Department of Otolaryngology Phılıps van Leydenlaan 15, Nıjmegen the Netherlands

1979, May 21–23 The Second Internatıonal Symposıum on Otıtıs Medıa wıth Effusıon wıll be held at Ohıo State Unıversıty, Columbus, Ohıo Addr Davıd J Lım, M D , Department of Otolaryngology Ohıo State Unıversıty Hospıtals, 456 Clınıc Drıve, Columbus, Ohıo 43210 USA